SORGHUM GENE EXPRESSION MODULATED BY WATER DEFICIT AND COLD STRESS

A Thesis

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SANGHYUN LIM

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

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Approved by:

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ABSTRACT

Sorghum Gene Expression Modulated by

Water Deficit and Cold Stress. (December 2006)

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Global gene expression in Sorghum bicolor, an important crop showing drought tolerance in arid and semi-arid cultivated areas, was monitored to exposure of 8-days seedlings to water deficit (20% polyethylene glycol) or cold stress (4 ºC). A sorghum cDNA microarray, including ~13,000 (milestone version 1) or ~28,000 (milestone version 2) unigenes, was used to examine gene expression in shoots and roots at 3 and 27hours after stress treatment. ~1,300 and ~2,300 genes were modulated by water deficit and cold stress, respectively. Up-regulated genes included previously identified stress-induced genes such as early drought-induced gene, dehydrin, late embryogenesis abundant gene, glycin and proline-rich gene, and water stress-inducible genes as well as unknown genes. Genes involved in signal transduction, lipid metabolism, transporter, and carbohydrate metabolism are induced. Quantitative real-time PCR was used to quantify changes in relative mRNA abundance for 333 and 108 genes in response to water deficit and cold stress, respectively. Stress-induced genes were classified by kinetics. Eighteen of 108 cold-induced genes were modulated by cold but not by ABA

and PEG treatment. This research provides the starting point for detailed analysis and comparison of water deficit and cold modulated gene networks in sorghum.

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INTRODUCTION

Water-deficit related stresses induced by drought and low temperature limit plant productivity in cultivated areas. Drought stress reduces the amount of available water for plant growth and development. Cold stress can result in water deficit through chilling injury which causes a phase transition from the liquid state to the gel crystalline state in cellular membranes (1). In response to dehydration stress, plants acquire tolerance by expressing stress inducible genes. Studies of gene expression in plants exposed to water deficit have identified numerous genes such as *responsive to dehydration* (*rd*), *early responsive to dehydration* (*erd*), and genes that encode late embryogenesis-abundant proteins (LEA), non specific lipid transfer proteins (LTP), and water channel proteins (2).

Cold-regulated (COR) genes involved in cold acclimation are being isolated in *Arabidopsis* and designated *lti* (low-temperature inducible), *kin* (cold-inducible), and *rd*. cor15a, cor78, rd29A, and kin1 of Arabidopsis have the C-repeat (CRT)/dehydration responsive element (DRE)-related motifs on their own promoter (3). Using a yeast one-hybrid system based on CRT/DRE motif, C-repeat/DRE binding factor 1 (CBF1) was isolated from Arabidopsis (4) and Gilmour *et al*., (5) isolated two additional homologs, CBF2, and CBF3.

This thesis follows the style and format of *Current Opinion in Plant Biology*.

Jaglo-Ottosen et al., (6) made transgenic Arabidopsis that overexpress CBF1. Transgenic plants and 5-day cold acclimated wild-type plants survived at -5 ºC for 2days whereas non-acclimated wild-type plants did not survive this treatment. The expression of COR genes in cold-acclimated and transgenic plants are correlated with cold tolerance. CBF-expressing transgenic Arabidopsis also showed enhanced tolerance to cold stress by affecting expression of COR genes and sugar metabolism (7).

Many COR genes were isolated in *B. napus*, *H. vulgaris* and *Medicago sativa* (8) and two different CBF-like proteins have been isolated in *B. napus*, wheat, rye, and tomato, LeCBF1 and LeCBF2 (9). Heish *et al*., (10) transferred Arabidopsis CBF1 into tomato plants. Transgenic tomatoes showed elevated tolerance to chilling stress by measuring chlorophyll fluorescence, ion leakage, and survival rate. In contrast to LeCBF1, LeCBF2 was not responsive to cold, but only high salinity and ABA treatment.

Microarray technology is a useful tool for genome-scale analysis of gene expression (11). Changes in gene expression that occur in response to abiotic stress such as drought (2, 12, 13), cold (2, 14), and salt (15, 16) have been characterized using microarrays in Arabidopsis (2), maize (15), barley (17), and rice (18). Transcriptome analysis under drought and cold stresses identified differences and cross-talk between these stress signaling cascades. Rabbani *et al*., (18) using a 1,700 rice element cDNA array identified 36 and 62 genes that were induced selectively by cold, and drought stresses, respectively, and 15 genes that were responsive to both conditions. The experiment showed that only 51 of 73 stress-inducible rice genes were also reported as

stress responsive in Arabidopsis, suggesting that rice may have different abiotic stress tolerance mechanisms.

Some water stress-inducible genes are induced by both drought and cold stress whereas others are modulated only by drought or by just cold stress. Under both drought and cold stresses, plants increase expression of RD29a (responsive to dehydration29a) which contains a dehydration-responsive *cis*-element (DRE) in its promoter (19). Analysis of the promoter of RD29B indicated that two ABA responsive elements (ABREs) are necessary for expressing the gene in response to drought or abscisic acid treatment (ABA) (20). Similarly, the cold-induced gene BN115 in winter *Brassica napus* has a low-temperature-responsive element (LTRE) in its 5'-proximal region that is required for this gene's expression (21).

The analysis of promoter sequences of stress-inducible genes identified by microarray analysis can identify potential *cis*-acting elements involved in stress-responsive gene expression. The analysis of potential *cis*-acting elements common to drought- and cold-inducible genes may help elucidate regulatory gene networks that mediate responses to drought and cold (22). It is well known that ABRE and DRE/C-repeat (CRT) are involved in drought- and cold-inducible gene expression (23). ABA controls drought-, and cold-inducible genes indicating that stress-inducible genes are regulated by ABA-dependent and ABA–independent pathways. Interestingly, many cold-inducible genes are regulated by an ABA-independent pathway (22). DRE/CRT is a major cis-acting element that mediates ABA-independent gene expression in response to drought and cold. cDNA encoding DRE-binding proteins (DREBs) and C-repeat-binding

factors (CBFs) that bind to DRE/CRT motifs were isolated using a yeast one-hybrid screen (24). Overexpression of DREB1/CBF driven by the 35S CaMV promoter in transgenic plants conferred increased tolerance to drought and cold stress (25, 26). EST analysis and GeneChip microarray experiments identified more than 40 genes downstream of DREB1/CBF consistent with the existence of a large DRE/DREB regulon (27). Surprisingly, some of the genes modulated in plants over expressing DREB did not contain a DRE motif in their promoters suggesting these genes were part of secondary cold stress response pathways.

Water stress is a major factor limiting agricultural production in the arid and semi-arid tropical regions of the world. Sorghum is one of the most important cereals in these areas because it has unusual tolerance to hot and dry environments. Sorghum has beneficial genes for agriculture and is a useful species for the comparative analysis of grass genes because it has a relatively small genome (750-890 million base pairs) (28). Comparison of drought- and cold-inducible genes in sorghum, maize and rice by comparative genomics is useful for the identification of conserved genes and regulatory elements and for understanding differences in tolerance among these species (29).

In this study, I propose to utilize sorghum cDNA microarrays to identify drought- and cold-inducible genes and confirm stress-induced genes by qRT-PCR.

MATERIALS AND METHODS

Water Deficit and Low Temperature Treatments

Eight day old seedlings of Sorghum bicolor cultivar BTx623 grown hydroponically under constant aeration were treated with polyethylene glycol (PEG) to induce water deficit or transferred to a 4°C growth chamber to induce cold stress. Previous studies in other species like Arabidopsis (2) and rice (18) used similar treatment to impose these stresses. The relative water content (RWC) of PEG treated plants decreased ~ 10%, 14%, and 30% following a 3 hr treatment with 5%, 10%, and 20% PEG. Cold-sensitive species like maize, rice, and tomato suffer chilling injury at low temperatures in the range of 0°C to 12°C (1). COR (cold-regulated) and CBF (CRT-binding factor) genes involved in the cold signaling pathway in tomato (14) and rice (18) are induced at 4°C. Therefore, sorghum seedlings were exposed to 4°C to induce low temperature stress. However, sorghum seedlings were able to recover after a 27hr treatment with 20% PEG or 4°C.

Sorghum cDNA microarrays

A collaborative sorghum genomics team (Pratt LH *et al*., University of Georgia; TAMU sorghum genomics group) constructed more than 14 cDNA libraries prepared from different tissues, developmental stages and plants subjected to abiotic and biotic stress. From these libraries, 117,682 ESTs were sequenced and 16,801 unique gene sequence contigs were identified based on analysis of 3’-sequences derived from the

cDNAs. 12,982 members of the unigene set were successfully amplified by PCR and used to generate sorghum cDNA microarrays (30).

Shoot and root tissues of 10 seedlings were harvested from 6 different biological replicates at 3 and 27 hour time points. RNA was extracted from each set of stress-treated and control plants. Microarrays were hybridized with cy3- and cy5- labeled cDNA probes generated from RNA isolated from shoots or roots. Hybridization experiments were replicated from the same RNA samples of each biological replicate to reduce variability from technical aspects such as probe generation, and slide to slide variation. Previous experiments revealed that analysis of 6 hybridizations or more allows on average ~9 false positives per experiment (0.07%) with showed signal variation more than 2 fold in control vs. control experiments (30). Therefore, I analyzed microarray data of at least 6 replicates obtained from at least 3 independent biological experiments to minimize biological and technical variability.

Drought- and Cold-Inducible Genes Identified by Microarray Analysis and Verified by qRT-PCR

Microarray data analysis revealed that drought and cold stresses caused differential expression of many sorghum genes. The transcripts of 1347 (10.4%) genes increased more than two fold following PEG treatment in at least one time point or one tissue. The dehydrins were highly induced in response to PEG or ABA treatment. These proteins have been proposed to act as water attractants or to have chaperone-like activities (31). Other genes activated by water deficit encoded late embryogenesis-

abundant proteins (LEA), heat-shock proteins, proline-rich proteins, water stress-inducible proteins (WSI), water-channel proteins, senescence-associated proteins and detoxification enzymes. Also induced were genes encoding many regulatory proteins that may regulate gene expression or other responses to water deficit. These include various protein kinases, protein phosphatases, zinc finger transcription factors (TF), and MAP kinases.

Microarray data can be associated with high background, and cross-hybridization of multigene family members. This can cause inconsistent results between microarray and qRT-PCR analyses (30). Rabbani *et al*. reported around 50% agreement between cDNA microarrays and RT-PCR assays (18). In the current study, expression of 184 (70%) of 263 genes modulated by PEG from microarray analysis was also confirmed by qRT-PCR. The difference of gene expression under different harvest time points, and tissues suggests that multiple regulatory systems modulate induction depending on time points and tissues. The result from qRT-PCR showed that many drought-inducible genes were induced following ABA application implying overlap and possible cross-talk between water deficit and ABA treatment.

Shinozaki *et al*. reported that most drought-inducible genes are also induced by ABA. However, they found only 10% of drought-inducible genes are induced under cold stress. In addition, ABA involved in osmotic stress-inducible gene expression is not a crucial factor in the expression of cold-inducible genes (12, 13, 23).

In this study, sorghum cDNA microarray analysis revealed that 15 (8.7%) of 172 of the drought-inducible genes with the highest expression are induced under cold stress.

These results are consistent with other reports in Arabidopsis (12), and rice (18). Under cold stress, cDNAs encoding many regulatory proteins and proteins that function in stress tolerance were induced. Interestingly, a gene encoding a calmodulin-like protein was highly induced at 3 and 27hr in shoots and roots by low temperature but not by PEG or ABA treatment. qRT-PCR was used to confirm that several genes were selectively activated by low temperature implying the existence of different *cis*-acting elements involved in each stress.

RESULTS

Microarray Analysis for Cold-Treated Sorghum Seedlings

Exposure of seedlings to 4 °C modulated expression of a large number of genes (Table 1).

Table 1. Number of genes showing changes in expression.

	Induced					Repressed		
Treatment	> 8	> 4	> 3	> 2.5	> 2	< 0.35	< 0.4	< 0.5
cold 3r	0	8	30	75	348	8	22	116
cold 27r	30	118	195	286	455	92	165	417
cold 27s	27	83	148	236	524	46	79	266

464, 872, and 790 genes were modulated at least 2-fold in cold-treated roots after 3 h, 27h, and in shoots after 27 h, respectively.

Up-Regulated Transcripts in Cold-Treated Roots

The list of cold-induced transcripts at 3h and 27h are given in Tables 2 and 3, respectively. Highly up-regulated transcripts at the 3h time point under cold stress were tentatively identified by Blast analysis as involved in stress responses, carbohydrate metabolism, signal transduction and transporters.

Table 2. List of cold-induced gene expressed at 3h time point in roots.

Accession number	Annotation	cold 3r
DSAF1_33_E06_A011	Calmodulin-like protein [Pennisetum ciliare]	7.2
IP1_9_H07_A002	P0683F02.7 protein (OJ1402_H07.15 protein) [Oryza sativa]	6.8
PIC1_68_C06_A002	Putative serine/threonine phosphatase type 2c [S. stapfianus]	5.9
WS1_100_F10_A002	P0039H02.8 protein [Oryza sativa]	5.3
LG1_228_A12_A002	Senescence-associated protein 5 [Hemerocallis cultivar]	5.1
WS1_68_C06_A002	protein WSI724 [Oryza sativa]	4.3
WS1_1_C03_A002	Hypothetical 10.9 kDa protein [Oryza sativa]	4.0
WOUND1_15_H04_A002	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	4.0
OX1_6_F10_A002	hypothetical protein; protein id: At1g19990.1 [A. thaliana]	3.8
WS1_24_E11_A002	protein WSI724 [Oryza sativa]	3.7
PI1_2_A07_A002	P0660F12.31 protein (P0614D08.28 protein) [Oryza sativa]	3.7
FM1_15_B03_A003	probable lipase [Zea mays]	3.6
WS1_37_D03_A002	aconitate hydratase [Lactococcus lactis]	3.6
POL1_62_G01_A002	hexose transporter [Hordeum vulgare]	3.6
DSBF1_30_E06_A010	Putative late embryogenesis abundant protein [Oryza sativa]	3.6
RHIZ2_16_C11_A003	glyceraldehyde-3-phosphate dehydrogenase [Zea mays]	3.4
PIC1_69_A07_A002	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	3.4
WS1_71_E05_A002	Senescence-associated protein 12 [Hemerocallis cultivar]	3.4
RHOH1_17_C11_A002	hypothetical protein F6N23.17 [Arabidopsis thaliana]	3.3
LG1_225_B11_A002	Putative amino acid transport protein [Arabidopsis thaliana]	3.3
RHIZ1_5_E03_A001	protein WSI724 [Oryza sativa]	3.3
WS1_63_G09_A002	P0022B05.9 protein [Oryza sativa]	3.3
POL1_62_H01_A002	O-methyltransferase ZRP4 (EC 2.1.1.-) (OMT) [Zea mays]	3.2
DSAF1_36_G09_A011	contains ESTs C96778(C10671), [Oryza sativa]	3.2
WS1_4_C09_A002	Xet1 protein [Schedonorus pratensis]	3.2
RHOH1_3_A10_A002	probable phytochelatin synthetase [Arabidopsis thaliana]	3.2
IP1_69_F01_A002	Caffeoyl CoA O-methyltransferase [Zea mays]	3.1
WOUND1_49_H08_A002	nucleic acid-binding protein [Zea mays]	3.1
WS1_4_B12_A002	Plasma membrane integral protein ZmPIP1-4 [Zea mays]	3.0
WS1_75_C12_A002	ABA-responsive protein homolog T31B5.20 [A. thaliana]	3.0
PIC1_54_F12_A002	P0638D12.11 protein [Oryza sativa]	3.0
RHIZ2_24_F05_A003	plasma membrane intrinsic protein BPW1 [Hordeum vulgare]	3.0
WS1_15_C10_A002	probable submergence induced protein 2A [O .sativa]	3.0
DG1_66_D01_A002	AT3g47620/F1P2_170 [Arabidopsis thaliana]	2.9
IP1_41_H10_A002	unnamed protein product [Zea mays]	2.9
OV1_20_C09_A002	protein WSI724 [Oryza sativa]	2.9
RHIZ2_8_C04_A003	Osmotin-like protein [Benincasa hispida]	2.9
LG1_214_B03_A002	IDS3 [Hordeum vulgare]	2.9
LG1_222_E02_A002	Putative ribosomal protein L12 [Oryza sativa]	2.9
PH1_17_D07_A002	Protein kinase-like protein [Oryza sativa]	2.8
WS1_30_B10_A002	B2-aldehyde-forming enzyme [Schizophyllum commune]	2.8

Table 2. Continued.

Accession number	Annotation	cold 3r
PIC1_11_G09_A002	P0683F02.18 protein (P0489B03.1 protein) [Oryza sativa]	2.8
PIC1_72_F04_A002	dehydration-induced protein (ERD15) [Arabidopsis thaliana]	2.7
OV1_29_F09_A002	protein F33E11.1 [imported] [Caenorhabditis elegans]	2.7
DSAF1_10_D09_A011	hypothetical protein T10G3.2 [Caenorhabditis elegans]	2.7
PIC1_4_D11_A002	P0492F05.26 protein (P0443E07.18 protein) [Oryza sativa]	2.7
EM1_71_A07_A002	60S ribosomal protein L3 [Oryza sativa]	2.7
WS1_67_C11_A002	P0460C04.15 protein [Oryza sativa]	2.7
LG1_282_D11_A002	P0666G04.13 protein [Oryza sativa]	2.7
LG1_223_C10_A002	Hypothetical protein [Oryza sativa]	2.7
FM1_15_H10_A003	Putative UDP-glucose dehydrogenase [Oryza sativa]	2.7
PI1_13_F07_A002	Phosphoinositide-dependent protein kinase [M musculus]	2.7
PIC1_5_H02_A002	Cell division control protein 13 [Saccharomyces cerevisiae]	2.7
RHIZ2_60_H06_A003	xyloglucan endo-1,4-beta-D-glucanase [Hordeum vulgare]	2.7
IP1_68_B10_A002	hypothetical protein, unlikely [Trypanosoma brucei]	2.7
PIC1_53_F04_A002	Sodium-calcium exchanger [Caenorhabditis elegans]	2.6
WOUND1_39_A07	Rad6 (Ubiquitin-conjugating enzyme E2) [Oryza sativa]	2.6
DG1_31_C05_A002	Hypothetical 32.9 kDa protein [Arabidopsis thaliana]	2.6
OX1_55_H12_A002	B2-aldehyde-forming enzyme [Schizophyllum commune]	2.6
LG1_224_G06_A002	Putative acetyl-CoA carboxylase [Oryza sativa]	2.6
DG1_70_D08_A002	putative cell wall biogenesis protein [S. pombe]	2.6
OV1_17_B06_A002	IDS3 [Hordeum vulgare]	2.6
PI1_34_E02_A002	fructose-bisphosphate aldolase [Arabidopsis thaliana]	2.6
IP1_54_D02_A002	hypothetical protein F9K21.120 [Arabidopsis thaliana]	2.6
PIC1_45_F04_A002	hypothetical protein VCA0334 [imported] [Vibrio cholerae]	2.6
PIC1_55_B10_A002	protein F5O11.12 [imported] [Arabidopsis thaliana]	2.6
IP1_70_A05_A002	Plasma membrane integral protein ZmPIP1-4 [Zea mays]	2.6
DSBF1_23_A06_A010	probable lipase [Zea mays]	2.5
RHIZ2_36_B09_A003	Elongation factor 1 alpha [Saccharum cultivar]	2.5
EM1_79_H10_A002	ATP synthase D chain [A. thaliana]	2.5
IP1_54_D01_A002	NADH dehydrogenase subunit 4 [Tetrahymena pyriformis]	2.5
RHIZ2_4_G03_A003	hypothetical protein T6G15.100 [Arabidopsis thaliana]	2.5
WS1_74_C10_A002	Putative ripening-related protein [Vitis vinifera]	2.5
FM1_46_A08_A003	Putative polyprotein [Zea mays]	2.5
RHIZ2_16_D10_A003	Phospholipase-like protein [Oryza sativa]	2.5
DSAF1_33_G05_A011	1700054F22Rik protein [Mus musculus]	2.5
IP1_61_C01_A002	RH44902p [Drosophila melanogaster]	2.5
CCC1_54_B06_A007	Muc5b protein [Mus musculus]	2.5
OV1_27_F11_A002	hypothetical protein F12F6.9 [Caenorhabditis elegans]	2.4
LG1_310_H07_A002	hypothetical protein; protein id: At1g50000.1 [A. thaliana]	2.4
NIT1_17_A08_A002	Hypothetical protein [Arabidopsis thaliana]	2.4
RHIZ2_21_H12_A003	Alpha-tubulin 1 [Eleusine indica]	2.4
RHIZ2_63_E04_A003	FtsH-like protein Pftf precursor [Nicotiana tabacum]	2.4
RHIZ2_21_C03_A003	CG5151 protein (LD13176p) [Drosophila melanogaster]	2.4
FE1_26_H02_A002	P0413G02.21 protein [Oryza sativa]	2.4

Table 2. Continued.

Accession number	Annotation	cold 3r
WS1_1_F12_A002	40S ribosomal protein S6 [Oryza sativa]	2.4
OV2_28_B07_A002	D-type cyclin [Zea mays]	2.4
LG1_343_D04_A002	erythrocyte membrane protein 1 [Plasmodium falciparum]	2.4
FM1_12_D08_A003	40S ribosomal protein S4 [Zea mays]	2.4
FM1_45_B08_A003	Enolase 1 (2-phosphoglycerate dehydratase 1) [Zea mays]	2.4
PI1_34_B11_A002	4-coumarate-CoA ligase-like protein [Arabidopsis thaliana]	2.4
FM1_29_A07_A003	Putative metal-binding protein [Arabidopsis thaliana]	2.4
PH1_2_H08_A002	Hypothetical protein [Arabidopsis thaliana]	2.4
IP1_69_A06_A002	Nucleoside diphosphate kinase III [A. thaliana]	2.4
ABA1_11_E02_A012	1200010K03Rik protein [Mus musculus]	2.4
OV1_27_H09_A002	VsaA-like protein [Oryza sativa]	2.4
RHOH1_28_A09	Histone H2A [Petroselinum crispum]	2.4
RHIZ2_18_C09_A003	Elongation factor 1 alpha [Saccharum hybrid cultivar]	2.4
WOUND1_9_A01	Glutathione S-transferase GST 26 (EC 2.5.1.18) [Zea mays]	2.4
OX1_49_D02_A002	S-adenosylmethionine decarboxylase proenzyme [Zea mays]	2.4
IP1_67_H11_A002	Putative heme A farnesyltransferase homolog [Oryza sativa]	2.4
LG1_222_F02_A002	Yer154p [Ashbya gossypii]	2.4
LG1_386_E08_A002	Putative chalcone synthase [Oryza sativa]	2.4
SS1_40_B10_A012	P0524E08.16 protein [Oryza sativa]	2.4
WS1_95_D03_A002	Unknown [Streptococcus agalactiae]	2.4
WS1_75_F12_A002	P0432B10.23 protein [Oryza sativa]	2.4
OX1_10_A05_A002	Cytoadherence linked asexual protein 2 [P. falciparum]	2.4
OV1_30_C12_A002	Beta-glucan binding protein [Phaseolus vulgaris]	2.4
OV2_6_G02_A002	hypothetical protein F19F18.170 [Arabidopsis thaliana]	2.3
PI1_15_D12_A002	Putative FH protein interacting protein FIP1 [Oryza sativa]	2.3
OV2_31_B06_A002	hypothetical protein T4I9.20 [Arabidopsis thaliana]	2.3
WS1_96_A12_A002	P0524E08.6 protein [Oryza sativa]	2.3
DSBF1_6_E09_A010	Glycogen synthase kinase binding protein [Danio rerio]	2.3
IP1_52_G11_A002	Unknown protein [Oryza sativa]	2.3
WS1_91_D08_A002	60S ribosomal protein L10a-1 [Arabidopsis thaliana]	2.3
PIC1_53_F10_A002	Phosphate transporter related protein [T. acidophilum]	2.3
WS1_37_C05_A002	T12H1.14 protein [Arabidopsis thaliana]	2.3
LG1_228_E07_A002	golgi associated protein se-wap41 [Zea mays]	2.3
FM1_11_F08_A003	New-glue protein 3 precursor [D. melanogaster]	2.3
FM1_1_G08_A003	unknown protein, 11341-13243 [imported] [A. thaliana]	2.3
PIC1_31_A07_A002	probable leucine-rich-repeat protein At2g19330 [A. thaliana]	2.3
IP1_3_D06_A002	Putative S-receptor kinase [Oryza sativa]	2.3
PIC1_11_F10_A002	OSJNBb0012E08.10 [Oryza sativa]	2.3

Table 3. Up-regulated transcripts in roots after 27h of cold treatment.

GenBank #	Annotaion	cold 27r
BE593348	P0039H02.8 protein [Oryza sativa]	17.5
CF769638	Abscisic acid response protein [Cucumis melo]	16.9
CF758554	Calmodulin-like protein [Pennisetum ciliare]	15.0
AW679099	S-adenosylmethionine decarboxylase 2 [Oryza sativa]	14.8
BM330997	Putative serine/threonine phosphatase type 2c [S. stapfianus]	14.8
CF490071	hexose transporter [Hordeum vulgare]	14.4
CF771697	probable lipase [Zea mays]	14.0
AW678861	Hypothetical 10.9 kDa protein [Oryza sativa]	13.0
AW747477	protein WSI724 [Oryza sativa]	11.7
CN140430	filamentous flower protein FIL [validated] [Arabidopsis thaliana]	11.6
CF490081	O-methyltransferase ZRP4 (EC 2.1.1.-) (OMT) [Zea mays]	11.4
AW747775	probable lipase [Zea mays]	11.3
BG410820	farnesylated protein 1 [Hordeum vulgare]	11.2
CN137136	B2-aldehyde-forming enzyme [Schizophyllum commune]	11.2
BE358724	Hypothetical 32.9 kDa protein [Arabidopsis thaliana]	11.0
AI724496	Hypothetical 66.5 kDa protein [C. elegans]	10.4
CN143299	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	9.9
CF757081	unnamed protein product [Zea mays]	9.8
BE363815	Senescence-associated protein 12 [Hemerocallis hybrid cultivar]	9.6
AI724571	protein WSI724 [Oryza sativa]	9.4
AW679458	protein WSI724 [Oryza sativa]	9.4
CN132454	hypothetical protein; protein id: At1g19990.1 [Arabidopsis thaliana]	9.0
AW679667	B2-aldehyde-forming enzyme [Schizophyllum commune]	8.1
AW747687	ABA-responsive protein homolog T31B5.20 [Arabidopsis thaliana]	8.0
BM331054	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	7.8
AW563464	Senescence-associated protein 5 [Hemerocallis hybrid cultivar]	7.6
CF771427	Hypothetical protein from PAC P0001A07 [Oryza sativa]	7.4
CF756127	trehalose-6-phosphate synthase homolog T13D8.4 [A. thaliana]	7.3
AW677982	unknown protein; protein id: At3g24100.1 [Arabidopsis thaliana]	7.2
BG049463	protein WSI724 [Oryza sativa]	7.0
BG357411	Protein translation factor SUI1 homolog (GOS2 protein) [Zea mays]	6.9
CF761779	Putative tetratricopeptide repeat protein [Oryza sativa]	6.6
BF480926	probable lipase [Zea mays]	6.5
BM331112	GTP binding protein-like [Arabidopsis thaliana]	6.5
BE592336	B1156H12.22 protein (OSJNBa0024F24.10 protein) [Oryza sativa]	6.5
AW679302	Hypothetical protein [Arabidopsis thaliana]	6.4
BE362310	Putative ripening-related protein [Vitis vinifera]	6.3
AW678973	unnamed protein product [Zea mays]	6.3
BM331104	Putative beta-glucosidase homolog [Oryza sativa]	6.0
BG052549	Unknown protein (Hypothetical protein) [Oryza sativa]	5.9
CN148074	probable RING zinc finger protein T23K23.8 [imported] [A. thaliana]	5.9
BE355117	Hypothetical protein (OSJNBA0036E02.8 protein) [Oryza sativa]	5.9
CF769580	beta-D-galactosidase [Streptococcus thermophilus]	5.8
BG158709	Senescence-associated protein 5 [Hemerocallis hybrid cultivar]	5.8

Table 3. Continued.

GenBank #	Annotaion	cold 27r
BE366362	Putative ribonucleoprotein [Oryza sativa]	5.8
CF770866	Putative zinc-finger helicase [Oryza sativa]	5.7
AW922491	P0018C10.2 protein (P0471B04.10 protein) [Oryza sativa]	5.7
CF755963	Early drought induced protein [Oryza sativa]	5.6
CF428678	Protein kinase-like protein [Oryza sativa]	5.6
AW679855	Hypothetical 10.5 kDa protein (Fragment) [Phleum pratense]	5.5
BE600380	Putative fatty acid condensing enzyme CUT1 [Oryza sativa]	5.5
CF771990	Thioredoxin-like protein TXL [D. melanogaster]	5.5
AW747518	Disease resistance protein RPM1 homolog [Sorghum bicolor]	5.5
BG933241	Protein translation factor SUI1 homolog (GOS2 protein) [Zea mays]	5.4
BI074459	Agglutinin Isolectin Vi [Urtica dioica]	5.4
AW677981	Putative embryo-specific protein [Oryza sativa]	5.4
BM329000	Unknown protein (Hypothetical protein) [Oryza sativa]	5.3
BM327868	GTP cyclohydrolase II (EC 3.5.4.25) [Arabidopsis thaliana]	5.3
BG649218	Thiazole biosynthetic enzyme 1-2, chloroplast precursor [Zea mays]	5.3
BF705200	Putative type-1 pathogenesis-related protein [Oryza sativa]	5.3
BE593721	Expressed protein (At2g02370/T16F16.16) [A. thaliana]	5.2
BM327134	Putative ribonucleoprotein [Oryza sativa]	5.2
BE355255	P0480E02.2 protein [Oryza sativa]	5.2
BE364693	Metalloproteinase inhibitor precursor [Bothrops jararaca]	5.2
AI724809	Phi-1 protein [Nicotiana tabacum]	5.2
BG946975	Putative ATP phosphoribosyl transferase [Oryza sativa]	5.2
BG240983	Protein translation factor SUI1 homolog (GOS2 protein) [Zea mays]	5.1
CF772192	Putative late embryogenesis abundant protein [Oryza sativa]	5.1
CD462387	protein WSI724 [Oryza sativa]	5.0
AI724499	ribosomal protein L23 [Zea mays]	5.0
BE363764	Protein translation factor SUI1 homolog (GOS2 protein) [Zea mays]	4.9
BG049201	Putative histone H2A [Oryza sativa]	4.9
BG357555	Protein disulfide isomerase precursor (PDI) (EC 5.3.4.1) [Zea mays]	4.9
AW747676	Biotin synthase (EC 2.8.1.6) [Arabidopsis thaliana]	4.8
AW747622	Putative ripening-related protein [Vitis vinifera]	4.8
BG556526	ESTs AU093391(E60370) [Oryza sativa]	4.8
BF421783	New-glue protein 3 precursor (NG-3) [Drosophila melanogaster]	4.8
AW747673	Putative cytochrome p450 [Oryza sativa]	4.7
BI351474	Ankyrin-like protein [Oryza sativa]	4.7
BM329717	Polyubiquitin [Sporobolus stapfianus]	4.6
BI245450	chaperonin gamma chain - like protein At5g26360.1 [A. thaliana]	4.6
BG948139	P0683F02.7 protein (OJ1402_H07.15 protein) [Oryza sativa]	4.5
CD223122	ESTs AU093391(E60370) [Oryza sativa]	4.5
BE918250	Protein translation factor SUI1 homolog (GOS2 protein) [Zea mays]	4.5
AW680111	Drought inducible 22 kDa protein [Saccharum officinarum]	4.5
AW679418	Hypothetical 10.5 kDa protein (Fragment) [Phleum pratense]	4.4
BM329373	P0410E01.4 protein (P0046B10.17 protein) [Oryza sativa]	4.4
CF756964	Glucose-6-phosphate 1-dehydrogenase [Treponema pallidum]	4.4

Table 3. Continued.

GenBank #	Annotaion	cold 27r
BI099424	unnamed protein product [Zea mays]	4.4
AW745843	Hypothetical protein [Arabidopsis thaliana]	4.3
AW924869	hypothetical protein F8K7.22 [Arabidopsis thaliana]	4.3
BG158189	probable Na+/H+ antiporter [imported] [Arabidopsis thaliana]	4.3
AW747533	Hypothetical protein [Arabidopsis thaliana]	4.3
BG357465	P0679C08.26 protein [Oryza sativa]	4.2
CF426886	Putative receptor kinase [Oryza sativa]	4.2
BE363768	Orf122 (Fragment) [Chlorobium tepidum]	4.2
AW678315	F16B3.4 protein [Arabidopsis thaliana]	4.2
BE355459	Peptidase-like protein [Oryza sativa]	4.2
BG049895	DNA helicase [Arabidopsis thaliana]	4.1
BM329731	hypothetical protein F23N20.8 [imported] [Arabidopsis thaliana]	4.1
BG158564	AT4g11790/T5C23_220 [Arabidopsis thaliana]	4.1
AW679798	hypothetical protein F7H2.1 [Arabidopsis thaliana]	4.1
BE363720	Protein translation factor SUI1 homolog (GOS2 protein) [Zea mays]	4.1
BM329083	polyubiquitin [Zea mays]	4.0
AW284904	acid phosphatase (EC 3.1.3.2) purple 2 [Ipomoea batatas]	4.0
BE355259	Putative chloroplast nucleoid DNA-binding protein [B. oleracea]	4.0
AW745588	Protein translation factor SUI1 homolog (GOS2 protein) [Zea mays]	3.9
AW681095	ESTs AU082454(S3638) [Oryza sativa]	3.9
BE363409	Putative chloroplast nucleoid DNA-binding protein [Oryza sativa]	3.9
BG239892	Beta-glucan binding protein [Phaseolus vulgaris]	3.8
AW746272	Putative chitinase III [Oryza sativa]	3.8
BE355116	acetyl-coa acetyltransferase [Schizosaccharomyces pombe]	3.8
AW679259	Protein translation factor SUI1 homolog (GOS2 protein) [Zea mays]	3.8
BE593779	P0046E05.13 protein [Oryza sativa]	3.8
BM327542	Hypothetical 10.6 kDa protein [Klebsiella pneumoniae]	3.7
AW680395	Arg-rich protein [Gallid herpesvirus 2]	3.7
AW680743	probable lipid transfer protein [Oryza sativa]	3.7
AW747675	CDH1-D (Fragment) [Gallus gallus]	3.7
CN132445	Expressed protein (Hypothetical protein) [Arabidopsis thaliana]	3.7
AW747785	Chitinase-B (Fragment) [Sorghum halepense]	3.7
BG102518	Polyubiquitin [Sporobolus stapfianus]	3.7
BG410723	Unknown protein (Hypothetical protein) [Oryza sativa]	3.7
BE355652	similar to carboxylesterase [Oryza sativa]	3.7
AW745802	aconitate hydratase (EC 4.2.1.3) [imported] [Lactococcus lactis]	3.6
BG355800	polyubiquitin [Zea mays]	3.6
BE363230	Hypothetical 157.2 kDa protein [Sorghum bicolor]	3.6
AW679131	Protein translation factor SUI1 homolog (GOS2 protein) [Zea mays]	3.6
BG049657	S-adenosylmethionine decarboxylase 2 [Oryza sativa]	3.6
BM329046	Putative zinc finger transcription factor [Oryza sativa]	3.5

Table 4 shows clustering of cold-induced genes by function in roots at the 3h time point. Many genes modulated by water deficit such as WSI (water-stress inducible), LEA (late embryo abundant), osmotin-like gene and dehydration-induced genes (ERD 15) were induced by the low temperature treatment. A hexose transporter, amino acid transport gene, UDP-glucose dehydrogenase, fructose-bisphosphate aldolase, B2-aldehyde forming enzymes were also highly expressed. Transcripts for signal transduction such as calmodulin-like protein, serine/threonine phosphatase, and kinase-like protein are induced.

Table 4. Clustering by function for transcripts up-regulated by cold treatment in roots at 3h time point.

GB number	Annotation	Cold 3r
	Signal transduction	
CF758554	Calmodulin-like protein [Pennisetum ciliare]	7.2
BM330997	Putative serine/threonine phosphatase type 2c [S. stapfianus]	5.87
CF428678	Protein kinase-like protein [Oryza sativa]	2.84
BM317963	Phosphoinositide-dependent protein kinase-1 [M. musculus]	2.69
BI245642	Small GTP-binding protein RACBP [Oryza sativa]	2.28
BF656785	GATA-binding transcription factor-like protein [A. thaliana]	2.26
BM328289	Putative GTP-binding protein [Arabidopsis thaliana]	2.25
BM330410	serine/threonine kinase-like protein [Oryza sativa]	2.2
	Stress-related	
AW747477	protein WSI724 [Oryza sativa]	4.29
CF772192	Putative late embryogenesis abundant protein [Oryza sativa]	3.56
AI724571	protein WSI724 [Oryza sativa]	3.26
AW747687	ABA-responsive protein homolog T31B5.20 [A. thaliana]	2.99
BG049463	protein WSI724 [Oryza sativa]	2.89
BG053390	Osmotin-like protein [Benincasa hispida]	2.88
BM331550	dehydration-induced protein (ERD15) [A. thaliana]	2.75
BM328901	probable leucine-rich-repeat protein At2g19330 [A. thaliana]	2.3
AW677981	Putative embryo-specific protein [Oryza sativa]	2.2
	Lipid-related	
BF480926	probable lipase [Zea mays]	3.64

Table 4. Continued.

GB number	Annotation	Cold 3r
CF771697	probable lipase [Zea mays]	2.53
BG053019	Phospholipase-like protein [Oryza sativa]	2.46
CF433105	Fatty acyl CoA reductase [Triticum aestivum]	2.21
	Transporter-related	
CF490071	hexose transporter [Hordeum vulgare]	3.61
AW283112	Putative amino acid transport protein [Arabidopsis thaliana]	3.29
AW680189	Plasma membrane integral protein ZmPIP1-4 [Zea mays]	3.01
BG102084	plasma membrane intrinsic protein BPW1 [Hordeum vulgare]	2.98
BI351109	Plasma membrane integral protein ZmPIP1-4 [Zea mays]	2.56
BM330569	Phosphate transporter related protein [T. acidophilum]	2.32
BG273199	putative ABC transporter family protein [Oryza sativa]	2.25
BE359220	Tonoplast membrane integral protein ZmTIP2-1 [Zea mays]	2.21
	Cabohydrate-related	
BG053010	glyceraldehyde-3-phosphate dehydrogenase [Zea mays]	3.4
BF480986	Putative UDP-glucose dehydrogenase [Oryza sativa]	2.69
BE366564	fructose-bisphosphate aldolase (EC 4.1.2.13) [A. thaliana]	2.59
BF656386	Enolase 1 (2-phosphoglycerate dehydratase 1) [Zea mays]	2.39
BM329759	Glyceraldehyde 3-phosphate dehydrogenase [Zea mays]	2.29
BI074277	Putative UDP-glucose dehydrogenase [Oryza sativa]	2.29
AW283694	phosphogluconate dehydrogenase (decarboxylating) [Zea mays]	2.24
	Hightly-induced genes	
BG948139	P0683F02.7 protein (OJ1402_H07.15 protein) [Oryza sativa]	6.77
BE593348	P0039H02.8 protein [Oryza sativa]	5.32
AW563464	Senescence-associated protein 5 [Hemerocallis cultivar]	5.14
AW678861	Hypothetical 10.9 kDa protein [Oryza sativa]	4.01
CN143299	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	3.95
CN132454	hypothetical protein; protein id: At1g19990.1 [A. thaliana]	3.8
BE365923	P0660F12.31 protein (P0614D08.28 protein) [Oryza sativa]	3.65
AW745802	aconitate hydratase (EC 4.2.1.3) [Lactococcus lactis]	3.62
BM331054	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	3.39
BE363815	Senescence-associated protein 12 [Hemerocallis cultivar]	3.39
CN126428	hypothetical protein F6N23.17 [Arabidopsis thaliana]	3.31
BE363489	P0022B05.9 protein [Oryza sativa]	3.26
CF490081	O-methyltransferase ZRP4 (EC 2.1.1.-) (OMT) [Zea mays]	3.23
CF758806	contains ESTs C96778(C10671) [Oryza sativa]	3.21
AW680192	Xet1 protein [Schedonorus pratensis]	3.21
CN124153	probable phytochelatin synthetase [Arabidopsis thaliana]	3.19
BI351083	Caffeoyl CoA O-methyltransferase [Zea mays]	3.13
CN147378	nucleic acid-binding protein [Zea mays]	3.11

At the 27h time point, many genes involved in carbohydrate metabolism are up regulated. Table 5 shows the clustering by function of cold stress up-regulated genes in roots at the 27h time point.

Table 5. Clustering by function cold-induced genes expressed in roots at 27h time point.

GB number	Annotation	cold 27r	cold 3r
	Signal transduction		
CF758554	Calmodulin-like protein [Pennisetum ciliare]	15.03	7.20
BM330997	Putative serine/threonine phosphatase type 2c [S. stapfianus]	14.79	5.87
BM331112	GTP binding protein-like [Arabidopsis thaliana]	6.51	1.64
CN148074	probable RING zinc finger protein T23K23.8 [A. thaliana]	5.93	0.89
CF428678	Protein kinase-like protein [Oryza sativa]	5.58	2.84
CF426886	Putative receptor kinase [Oryza sativa]	4.18	1.19
BM329046	Putative zinc finger transcription factor [Oryza sativa]	3.54	1.02
AW679277	Phosphatidic acid phosphatase-like protein [L. donovani]	3.49	0.92
BE357647	MAP kinase-like protein [Oryza sativa]	3.42	1.14
BM317963	Phosphoinositide-dependent protein kinase-1 [M. musculus]	3.34	2.69
BE593730	Similar to PTK2 protein tyrosine kinase 2 [Homo sapiens]	3.27	1.26
BM329814	Putative calmodulin-binding protein [Oryza sativa]	3.24	1.18
BM331204	Protein phosphatase 2C-like protein [Oryza sativa]	3.01	1.77
	stress-related		
CF769638	Abscisic acid response protein [Cucumis melo]	16.91	1.88
AW747477	protein WSI724 [Oryza sativa]	11.71	4.29
CN137136	Putative glycine and proline-rich protein [Sporobolus stapfianus]	11.19	2.62
AI724571	protein WSI724 [Oryza sativa]	9.37	3.26
AW679458	protein WSI724 [Oryza sativa]	9.36	3.68
AW679667	Putative glycine and proline-rich protein [Sporobolus stapfianus]	8.07	2.77
AW747687	ABA-responsive protein homolog T31B5.20 [A. thaliana]	8.01	2.99
BG049463	protein WSI724 [Oryza sativa]	7.00	2.89
CF755963	Early drought induced protein [Oryza sativa]	5.61	1.36
AW677981	Putative embryo-specific protein [Oryza sativa]	5.38	2.20
CF772192	Putative late embryogenesis abundant protein [Oryza sativa]	5.07	3.56
CD462387	protein WSI724 [Oryza sativa]	5.03	1.38
AW680111	Drought inducible 22 kDa protein [Saccharum officinarum]	4.50	1.31
AW680743	probable lipid transfer protein [Oryza sativa]	3.73	0.82
BE592440	Leucine rich repeat protein, putative [Arabidopsis thaliana]	3.16	1.32
CN148252	Putative hydroxyproline-rich glycoprotein [Oryza sativa]	2.98	0.51
CD424449	Histidine-rich glycoprotein 2 [Rattus norvegicus]	2.91	0.94

Table 5. Continued.

GB number	Annotation	cold 27r	cold 3r
	Trasporter-related		
CF490071	hexose transporter [Hordeum vulgare]	14.39	3.61
BG158189	probable Na+/H+ antiporter [imported] [Arabidopsis thaliana]	4.30	1.98
CF772054	High-affinity potassium uptake transporter [D. occidentalis]	2.99	1.17
CD211889	dipeptide ABC transporter, permease protein [Helicobacter pylori]	2.79	0.96
CF771910	Solute carrier family 6 [Rattus norvegicus]	2.75	1.26
	Lipid-related		
CF771697	probable lipase [Zea mays]	14.03	2.53
AW747775	probable lipase [Zea mays]	11.35	1.23
BF480926	probable lipase [Zea mays]	6.52	3.64
BE600380	Putative fatty acid condensing enzyme CUT1 [Oryza sativa]	5.50	1.15
CF770555	Oxysterol-binding protein-like [Arabidopsis thaliana]	3.30	1.54
AI724605	putative triacylglycerol/steryl ester hydrolase [A. thaliana]	2.91	1.26
	ROS-related		
CF771990	Thioredoxin-like protein TXL [Drosophila melanogaster]	5.50	1.68
AW745838	Thioredoxin H-type 1 (TRX-H1) [Nicotiana tabacum]	2.74	1.83
	Carbohydrate-related		
CF756127	trehalose-6-phosphate synthase homolog T13D8.4 [A. thaliana]	7.28	2.05
BM331104	Putative beta-glucosidase homolog [Oryza sativa]	6.01	1.72
CF769580	beta-D-galactosidase [Streptococcus thermophilus]	5.83	1.14
CF756964	Glucose-6-phosphate 1-dehydrogenase [Treponema pallidum]	4.39	1.87
BM328161	glucose-6-phosphate/phosphate-translocator precursor [P .sativum]	3.29	1.08
	Highly-induced genes		
BE593348	P0039H02.8 protein [Oryza sativa]	17.55	5.32
AW679099	S-adenosylmethionine decarboxylase 2 [Oryza sativa]	14.85	1.31
AW678861	Hypothetical 10.9 kDa protein [Oryza sativa]	13.02	4.01
CN140430	filamentous flower protein FIL [Arabidopsis thaliana]	11.58	1.10
CF490081	O-methyltransferase ZRP4 (EC 2.1.1.-) (OMT) [Zea mays]	11.40	3.23
BG410820	farnesylated protein 1 [Hordeum vulgare]	11.24	1.46
CN137136	B2-aldehyde-forming enzyme [Schizophyllum commune]	11.19	2.62
BE358724	Hypothetical 32.9 kDa protein [Arabidopsis thaliana]	11.00	2.62
AI724496	Hypothetical 66.5 kDa protein [Caenorhabditis elegans]	10.39	1.82
CN143299	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	9.94	3.95
CF757081	unnamed protein product [Zea mays]	9.78	2.10
BE363815	Senescence-associated protein 12 [Hemerocallis cultivar]	9.65	3.39
CN132454	hypothetical protein; protein id: At1g19990.1 [A. thaliana]	8.96	3.80
AW679667	B2-aldehyde-forming enzyme [Schizophyllum commune]	8.07	2.77

Table 5. Continued.

GB number	Annotation	cold 27r	cold 3r
BM331054	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	7.81	3.39
AW563464	Senescence-associated protein 5 [Hemerocallis cultivar]	7.56	5.14
CF771427	Hypothetical protein from PAC P0001A07 [Oryza sativa]	7.38	1.27
AW677982	unknown protein; protein id: At3g24100.1 [A. thaliana]	7.22	1.37
AW678304	AT5g56980/MHM17_10 [Arabidopsis thaliana]	7.13	1.06
BG357411	Protein translation factor SUI1 homolog [Zea mays]	6.89	2.09
BE363489	P0022B05.9 protein [Oryza sativa]	6.65	3.26
CF761779	Putative tetratricopeptide repeat protein [Oryza sativa]	6.63	1.97
BE592336	B1156H12.22 protein (OSJNBa0024F24.10 protein) [O. sativa]	6.49	2.15
AW679302	Hypothetical protein [Arabidopsis thaliana]	6.40	2.14
BE362310	Putative ripening-related protein [Vitis vinifera]	6.34	1.67
AW678973	unnamed protein product [Zea mays]	6.28	0.73
BG052549	Unknown protein (Hypothetical protein) [Oryza sativa]	5.94	1.10
BE355117	Hypothetical protein (OSJNBA0036E02.8 protein) [O. sativa]	5.86	1.59
BG158709	Senescence-associated protein 5 [Hemerocallis cultivar]	5.79	1.94
BE366362	Putative ribonucleoprotein [Oryza sativa]	5.76	0.93
CF770866	Putative zinc-finger helicase [Oryza sativa]	5.74	1.96
AW922491	P0018C10.2 protein (P0471B04.10 protein) [Oryza sativa]	5.70	2.09
AW679855	Hypothetical 10.5 kDa protein (Fragment) [Phleum pratense]	5.51	1.06
AW747518	Disease resistance protein RPM1 homolog [Sorghum bicolor]	5.48	0.95
BG933241	Protein translation factor SUI1 homolog [Zea mays]	5.40	1.08
BI074459	Agglutinin Isolectin Vi [Urtica dioica]	5.39	0.64
BM329000	Unknown protein (Hypothetical protein) [Oryza sativa]	5.32	1.66
BM327868	GTP cyclohydrolase II (EC 3.5.4.25) [Arabidopsis thaliana]	5.31	2.01
BG649218	Thiazole biosynthetic enzyme 1-2 [Zea mays]	5.30	0.25
BF705200	Putative type-1 pathogenesis-related protein [Oryza sativa]	5.30	0.79
BE593721	Expressed protein (At2g02370/T16F16.16) [A. thaliana]	5.22	1.25
BM327134	Putative ribonucleoprotein [Oryza sativa]	5.20	1.09
BE355255	P0480E02.2 protein [Oryza sativa]	5.20	1.14
BE364693	Metalloproteinase inhibitor precursor [Bothrops jararaca]	5.17	1.35
AI724809	Phi-1 protein [Nicotiana tabacum]	5.16	1.46
BG946975	Putative ATP phosphoribosyl transferase [Oryza sativa]	5.16	1.46
BG240983	Protein translation factor SUI1 homolog [Zea mays]	5.07	1.27

Genes that show differences in time course of induction in response to low temperature are shown in Table 6. At least 3 groups of genes with different kinetics of induction were identified. Interestingly, calmodulin-like protein, serine/threonine phosphatase, water stress inducible genes, lipase, B2-aldehyde-forming enzyme and senescence-associated protein are highly expressed at both time points.

Table 6. Variation in kinetics of gene induction in response to low temperature.

GB number	Annotation	3r	27r
	high expression at 3r		
BE365923	P0660F12.31 protein (P0614D08.28 protein) [Oryza sativa]	3.65	1.44
BG053010	glyceraldehyde-3-phosphate dehydrogenase [Zea mays]	3.40	1.32
CN126428	hypothetical protein F6N23.17 [Arabidopsis thaliana]	3.31	0.79
AW283112	Putative amino acid transport protein [Arabidopsis thaliana]	3.29	0.68
CF758806	contains ESTs C96778(C10671) [Oryza sativa]	3.21	1.95
AW680192	Xet1 protein [Schedonorus pratensis]	3.21	1.44
CN124153	probable phytochelatin synthetase [Arabidopsis thaliana]	3.19	1.54
BI351083	Caffeoyl CoA O-methyltransferase (EC 2.1.1.104) [Zea mays]	3.13	1.83
CN147378	nucleic acid-binding protein [Zea mays]	3.11	0.79
AW680189	Plasma membrane integral protein ZmPIP1-4 [Zea mays]	3.01	0.36
BG102084	plasma membrane intrinsic protein BPW1 [Hordeum vulgare]	2.98	0.97
AW678394	probable submergence induced [Oryza sativa]	2.97	0.75
BE360776	AT3g47620/F1P2_170 [Arabidopsis thaliana]	2.94	0.63
BG053390	Osmotin-like protein [Benincasa hispida]	2.88	0.49
AW284675	IDS3 [Hordeum vulgare]	2.87	1.87
BM327302	P0683F02.18 protein (P0489B03.1 protein) [Oryza sativa]	2.76	1.24
BM331550	dehydration-induced protein (ERD15) [A. thaliana]	2.75	2.06
BG239839	protein F33E11.1 [imported] [Caenorhabditis elegans]	2.73	1.43
CF756856	hypothetical protein T10G3.2 [Caenorhabditis elegans]	2.72	0.75
BM326893	P0492F05.26 protein (P0443E07.18 protein) [Oryza sativa]	2.72	1.44
BG464540	60S ribosomal protein L3 [Oryza sativa]	2.71	1.12
AW747340	P0460C04.15 protein [Oryza sativa]	2.71	1.42
AW283918	P0666G04.13 protein [Oryza sativa]	2.71	1.29
AW283365	Hypothetical protein [Oryza sativa]	2.70	1.38
BF480986	Putative UDP-glucose dehydrogenase [Oryza sativa]	2.69	1.06
	high expression at 27r		
CF769638	Abscisic acid response protein [Cucumis melo]	1.88	16.91
AW679099	S-adenosylmethionine decarboxylase 2 [Oryza sativa]	1.31	14.85
CF771697	probable lipase [Zea mays]	2.53	14.03
CN140430	filamentous flower protein FIL [Arabidopsis thaliana]	1.10	11.58
AW747775	probable lipase [Zea mays]	1.23	11.35
BG410820	farnesylated protein 1 [Hordeum vulgare]	1.46	11.24
CN137136	B2-aldehyde-forming enzyme [Schizophyllum commune]	2.62	11.19
BE358724	Hypothetical 32.9 kDa protein [Arabidopsis thaliana]	2.62	11.00
AI724496	Hypothetical 66.5 kDa protein [Caenorhabditis elegans]	1.82	10.39

Table 6. Continued.

GB number	Annotation	3r	27r
CF757081	unnamed protein product [Zea mays]	2.10	9.78
CF771427	Hypothetical protein from PAC P0001A07 [Oryza sativa]	1.27	7.38
CF756127	trehalose-6-phosphate synthase [Arabidopsis thaliana]	2.05	7.28
AW677982	unknown protein: At3g24100.1 [Arabidopsis thaliana]	1.37	7.22
AW678304	AT5g56980/MHM17_10 [Arabidopsis thaliana]	1.06	7.13
BG357411	Protein translation factor SUI1 homolog [Zea mays]	2.09	6.89
CF761779	Putative tetratricopeptide repeat protein [Oryza sativa]	1.97	6.63
BM331112	GTP binding protein-like [Arabidopsis thaliana]	1.64	6.51
BE592336	B1156H12.22 protein [Oryza sativa]	2.15	6.49
AW679302	Hypothetical protein [Arabidopsis thaliana]	2.14	6.40
BE362310	Putative ripening-related protein [Vitis vinifera]	1.67	6.34
AW678973	unnamed protein product [Zea mays]	0.73	6.28
BM331104	Putative beta-glucosidase homolog [Oryza sativa]	1.72	6.01
BG052549	Unknown protein (Hypothetical protein) [Oryza sativa]	1.10	5.94
CN148074	probable RING zinc finger protein [Arabidopsis thaliana]	0.89	5.93
BE355117	Hypothetical protein [Oryza sativa]	1.59	5.86
CF769580	beta-D-galactosidase [Streptococcus thermophilus]	1.14	5.83
BG158709	Senescence-associated protein 5 [Hemerocallis cultivar]	1.94	5.79
BE366362	Putative ribonucleoprotein [Oryza sativa]	0.93	5.76
CF770866	Putative zinc-finger helicase [Oryza sativa]	1.96	5.74
AW922491	P0018C10.2 protein (P0471B04.10 protein) [Oryza sativa]	2.09	5.70
CF755963	Early drought induced protein [Oryza sativa]	1.36	5.61
AW679855	Hypothetical 10.5 kDa protein (Fragment) [Phleum pratense]	1.06	5.51
BE600380	Putative fatty acid condensing enzyme CUT1 [Oryza sativa]	1.15	5.50
CF771990	Thioredoxin-like protein TXL [Drosophila melanogaster]	1.68	5.50
AW747518	Disease resistance protein RPM1 homolog [Sorghum bicolor]	0.95	5.48
BG933241	Protein translation factor SUI1 homolog [Zea mays]	1.08	5.40
BI074459	Agglutinin Isolectin Vi [Urtica dioica]	0.64	5.39
AW677981	Putative embryo-specific protein [Oryza sativa]	2.20	5.38
BM329000	Unknown protein (Hypothetical protein) [Oryza sativa]	1.66	5.32
BM327868	GTP cyclohydrolase II (EC 3.5.4.25) [Arabidopsis thaliana]	2.01	5.31
BG649218	Thiazole biosynthetic enzyme 1-2 [Zea mays]	0.25	5.30
BF705200	Putative type-1 pathogenesis-related protein [Oryza sativa]	0.79	5.30
BE593721	Expressed protein (At2g02370/T16F16.16) [Arabidopsis thaliana]	1.25	5.22
BM327134	Putative ribonucleoprotein [Oryza sativa]	1.09	5.20
BE355255	P0480E02.2 protein [Oryza sativa]	1.14	5.20
BE364693	Metalloproteinase inhibitor precursor [Bothrops jararaca]	1.35	5.17
AI724809	Phi-1 protein [Nicotiana tabacum]	1.46	5.16
BG946975	Putative ATP phosphoribosyl transferase [Oryza sativa]	1.46	5.16
BG240983	Protein translation factor SUI1 homolog [Zea mays]	1.27	5.07
CD462387	protein WSI724 [Oryza sativa]	1.38	5.03
AW680111	Drought inducible 22 kDa protein [Saccharum officinarum]	1.31	4.50

Table 6. Continued.

GB number	Annotation	3r	27r
	high expression at both time point		
CF758554	Calmodulin-like protein [Pennisetum ciliare]	7.20	15.03
BG948139	P0683F02.7 protein (OJ1402_H07.15 protein) [Oryza sativa]	6.77	4.53
BM330997	Putative serine/threonine phosphatase type 2c [S. stapfianus]	5.87	14.79
BE593348	P0039H02.8 protein [Oryza sativa]	5.32	17.55
AW563464	Senescence-associated protein 5 [Hemerocallis hybrid cultivar]	5.14	7.56
AW747477	protein WSI724 [Oryza sativa]	4.29	11.71
AW678861	Hypothetical 10.9 kDa protein [Oryza sativa]	4.01	13.02
CN143299	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	3.95	9.94
CN132454	hypothetical protein; protein id: At1g19990.1 [A. thaliana]	3.80	8.96
AW679458	protein WSI724 [Oryza sativa]	3.68	9.36
BF480926	probable lipase [Zea mays]	3.64	6.52
AW745802	aconitate hydratase (EC 4.2.1.3) [imported] [Lactococcus lactis]	3.62	3.65
CF490071	hexose transporter [Hordeum vulgare]	3.61	14.39
CF772192	Putative late embryogenesis abundant protein [Oryza sativa]	3.56	5.07
BM331054	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	3.39	7.81
BE363815	Senescence-associated protein 12 [Hemerocallis hybrid cultivar]	3.39	9.65
AI724571	protein WSI724 [Oryza sativa]	3.26	9.37
BE363489	P0022B05.9 protein [Oryza sativa]	3.26	6.65
CF490081	O-methyltransferase ZRP4 (EC 2.1.1.-) (OMT) [Zea mays]	3.23	11.40
AW747687	ABA-responsive protein homolog T31B5.20 [Arabidopsis thaliana]	2.99	8.01
BM330650	P0638D12.11 protein [Oryza sativa]	2.98	2.66
BI099424	AP2-domain DNA-binding protein [Catharanthus roseus]	2.91	4.38
BG049463	protein WSI724 [Oryza sativa]	2.89	7.00
AW283704	Putative ribosomal protein L12 [Oryza sativa]	2.86	2.42
CF428678	Protein kinase-like protein [Oryza sativa]	2.84	5.58
AW679667	B2-aldehyde-forming enzyme [Schizophyllum commune]	2.77	8.07
BM317963	Phosphoinositide-dependent protein kinase-1 [Mus musculus]	2.69	3.34

Up-Regulated Transcripts in Cold-Treated Shoots

Genes modulated by low temperature in sorghum shoots are listed in Table 7.

Table 7. Genes modulated in shoots after 27h exposure to low temperature.

GB number	P3 target desc	Cold27s
	Signal transduction	
BM330997	serine/threonine phosphatase type 2c [S. stapfianus]	10.3
CF758554	Calmodulin-like protein [Pennisetum ciliare]	7.9
AW678870	OsNAC6 protein [imported] [Oryza sativa]	7.2

Table 7. Continued.

GB number	P3 target desc	Cold27s
BM331052	Receptor-like kinase [Hordeum vulgare]	6.8
BM329814	Putative calmodulin-binding protein [Oryza sativa]	5.6
BE593730	Similar to PTK2 protein tyrosine kinase 2 [Homo sapiens]	4.9
BM331112	GTP binding protein-like [Arabidopsis thaliana]	4.6
CF428678	Protein kinase-like protein [Oryza sativa]	3.3
	Stress-related	
CF769638	Abscisic acid response protein [Cucumis melo]	21.5
BM331550	dehydration-induced protein (ERD15) [A. thaliana]	12.0
AW747477	protein WSI724 [Oryza sativa]	6.4
CF755963	Early drought induced protein [Oryza sativa]	5.8
AW679458	protein WSI724 [Oryza sativa]	5.5
BE592440	Leucine rich repeat protein, putative [A. thaliana]	4.4
CF772192	Putative late embryogenesis abundant protein [O. sativa]	4.4
BE363779	Late embryogenesis abundant protein [A. thaliana]	4.1
AI724571	protein WSI724 [Oryza sativa]	4.0
BG049463	protein WSI724 [Oryza sativa]	3.9
	Carbohydrate-related	
CF756127	trehalose-6-phosphate synthase homolog [A. thaliana]	12.3
BM331104	Putative beta-glucosidase homolog [Oryza sativa]	8.7
CF769608	phosphoglucomutase precursor [Spinacia oleracea]	3.4
CF490071	hexose transporter [Hordeum vulgare]	3.2
	Lipid-related	
BE600380	Putative fatty acid condensing enzyme CUT1 [O. sativa]	9.3
CF771697	probable lipase [Zea mays]	4.9
BF480926	probable lipase [Zea mays]	3.4
	Transporter-related	
CD211889	dipeptide ABC transporter [Helicobacter pylori]	3.3
	ROS-related	
AW284125	Putative glutathione peroxidase (EC 1.11.1.9) [Zea mays]	3.5
BF422046	Superoxide dismutase [Cu-Zn] 4A [Zea mays]	3.5
BE595039	Oxidase [Oryza sativa]	3.4
	Highly-induced genes	
BM331054	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	38.4
AW678861	Hypothetical 10.9 kDa protein [Oryza sativa]	30.5
CN143299	P0005A05.30 protein (P0482C06.13 protein) [Oryza sativa]	27.5
AW679251	SMCP-2 [Homo sapiens]	22.8
CF757081	unnamed protein product [Zea mays]	21.3
BE593348	P0039H02.8 protein [Oryza sativa]	20.7

Table 7. Continued.

GB number	P3 target desc	Cold27s
AW678813	Putative selenium-binding protein [Oryza sativa]	20.3
AW679099	S-adenosylmethionine decarboxylase 2 [Oryza sativa]	19.5
BE358724	Hypothetical 32.9 kDa protein [Arabidopsis thaliana]	13.9
BG948139	P0683F02.7 protein (OJ1402_H07.15 protein) [O sativa]	12.6
BG649218	Thiazole biosynthetic enzyme 1-2 [Zea mays]	12.0
BE592436	Hypothetical protein [Arabidopsis thaliana]	11.5
AW678973	unnamed protein product [Zea mays]	11.4
BM329717	Polyubiquitin [Sporobolus stapfianus]	11.4
AW745559	AT07117p [Drosophila melanogaster]	11.3
CN132454	hypothetical protein; protein id: At1g19990.1 [A. thaliana]	10.1
BE363815	Senescence-associated protein 12 [Hemerocallis cultivar]	8.6
AW680025	similar to carboxylesterase [Oryza sativa]	8.2
BE592436	Hypothetical protein [Arabidopsis thaliana]	7.4
CN140430	filamentous flower protein FIL [Arabidopsis thaliana]	7.0
AI724496	Hypothetical 66.5 kDa protein [Caenorhabditis elegans]	6.7
AW747510	Hypothetical protein [Arabidopsis thaliana]	6.5
BG102518	Polyubiquitin [Sporobolus stapfianus]	6.5
BG158709	Senescence-associated protein 5 [Hemerocallis cultivar]	6.3
CF771989	pumilio-family RNA-binding protein [Arabidopsis thaliana]	6.2
BF656732	Chloroplast 30S ribosomal protein S11 [Zea mays]	6.0
AI724809	Phi-1 protein [Nicotiana tabacum]	5.9
AW563464	Senescence-associated protein 5 [Hemerocallis cultivar]	5.5
CF757163	B1097D05.23 protein [Oryza sativa]	5.5
AW747472	Thiazole biosynthetic enzyme 1-2 [Zea mays]	5.4
BM329083	polyubiquitin [Zea mays]	5.4
AW678346	polyubiquitin 6 [Oryza sativa]	5.2
AW747622	Putative ripening-related protein [Vitis vinifera]	5.0
BI099424	unnamed protein product [Zea mays]	5.0
CF758806	contains ESTs C96778(C10671) [Oryza sativa]	4.9
BE367025	cellulase E-4 [Thermobifida fusca]	4.8
BI074459	Agglutinin Isolectin Vi [Urtica dioica]	4.8
BM329410	cryptochrome 2 apoprotein [Sorghum bicolor]	4.7
AW680192	Xet1 protein [Schedonorus pratensis]	4.7
BM330297	Expressed protein [Arabidopsis thaliana]	4.6
BG103754	polyubiquitin [Zea mays]	4.6

Confirmation of Gene Expression by qRT-PCR

To confirm the expression of genes identified by microarray analysis, qRT-PCR was performed (Table 8). The expression of 69 (64%) genes was modulated at least 2-fold in one or more time point/tissue combinations.

Table 8. qRT-PCR assays for 108 transcripts expressed under cold treatment.

Clone name	3S	27S	3r	27r	Annotation
IP1_9_H07_A002	1.1	0.7	1.6	0.8	P0683F02.7 protein (OJ1402_H07.15 protein) [O sativa]
PI1_2_A07_A002	1.0	0.6	1.0	0.3	P0660F12.31 protein (P0614D08.28 protein) [O sativa]
RHIZ2_16_C11_A003	0.9	0.7	2.2	1.2	glyceraldehyde-3-phosphate dehydrogenase
RHOH1_17_C11_A002	0.8	1.0	1.1	0.6	hypothetical protein F6N23.17 [Arabidopsis thaliana]
LG1_225_B11_A002	2.9	9.3	2.0	7.4	Putative amino acid transport protein [A thaliana]
DSAF1_36_G09_A011	4.8	12.5	5.0	5.2	unknown protein [Oryza sativa]
WS1_4_C09_A002	2.6	5.1	14.4	9.1	Xet1 protein [Schedonorus pratensis]
RHOH1_3_A10_A002	0.8	0.5	1.5	0.3	probable phytochelatin synthetase [Arabidopsis thaliana]
WOUND1_49_H08_A002	0.5	0.7	1.5	2.0	nucleic acid-binding protein [Zea mays]
WS1_4_B12_A002	1.2	0.4	1.6	0.4	Plasma membrane integral protein ZmPIP1-4 [Zea mays]
RHIZ2_24_F05_A003	2.3	1.1	3.2	1.3	plasma membrane intrinsic protein BPW1 [H vulgare]
FM1_15_H10_A003	1.1	1.2	1.7	1.1	Putative UDP-glucose dehydrogenase [Oryza sativa]
PI1_34_E02_A002	2.5	1.1	5.0	6.3	fructose-bisphosphate aldolase [Arabidopsis thaliana]
IP1_70_A05_A002	1.6	0.7	1.3	0.9	Plasma membrane integral protein ZmPIP1-4 [Zea mays]
RHIZ2_16_D10_A003	1.5	1.3	1.0	1.2	Phospholipase-like protein [Oryza sativa]
FM1_29_A07_A003	1.2	0.8	1.4	0.5	Putative metal-binding protein [Arabidopsis thaliana]
WOUND1_9_A01_A002	1.0	1.2	5.1	4.5	Glutathione S-transferase GST 26 [Zea mays]
PI1_15_D12_A002	1.3	2.1	1.1	1.1	Plasma membrane integral protein ZmPIP1-4 [Zea mays]
RHIZ2_23_B05_A003	0.3	1.0	0.7	0.8	Phospholipase-like protein [Oryza sativa]

Table 8. Continued.

Clone name	3S	27S	3r	27r	Annotation
NIT1_25_D07_A002	0.6	0.5	1.4	1.0	Putative metal-binding protein [Arabidopsis thaliana]
DG1_39_E03_A002	2.0	1.0	1.8	0.7	Putative aquaporin (Fragment) [Oryza sativa]
WS1_100_F10_A002	2.6	17.5	13.2	38.2	P0039H02.8 protein [Oryza sativa]
DSBF1_2_H07_A010	6.5	35.0	6.4	80.0	Abscisic acid response protein [Cucumis melo]
WS1_1_C03_A002	4.7	31.7	16.5	26.8	Hypothetical 10.9 kDa protein [Oryza sativa]
OX1_36_D04_A002	0.7	1.3	0.6	0.0	filamentous flower protein FIL [Arabidopsis thaliana]
POL1_62_H01_A002	0.4	1.2	1.2	2.0	O-methyltransferase ZRP4 (EC 2.1.1.-) [Zea mays]
EM1_26_D02_A002	2.4	462.4	3.0	23.3	farnesylated protein 1 [Hordeum vulgare]
OX1_55_H12_A002	2.3	11.8	11.4	32.9	B2-aldehyde-forming enzyme [Schizophyllum commune]
WOUND1_15_H04_A002	7.6	20.4	4.6	15.6	P0005A05.30 protein (P0482C06.13 protein) [O. sativa]
DSAF1_13_D12_A011	4.0	19.7	5.4	17.2	unnamed protein product [Zea mays]
WS1_71_E05_A002	2.2	14.8	21.6	48.9	Senescence-associated protein 12 [H cultivar]
WS1_24_E11_A002	1.2	2.1	2.2	6.0	protein WSI724 [Oryza sativa]
OX1_6_F10_A002	0.8	0.8	1.5	0.6	hypothetical protein; At1g19990.1 [A. thaliana]
LG1_228_A12_A002	2.4	11.8	5.2	7.9	Senescence-associated protein 5 [Hemerocallis cultivar]
DSBF1_19_C01_A010	1.0	5.0	2.2	5.6	Hypothetical protein from PAC P0001A07 [Oryza sativa]
OV1_20_C09_A002	1.3	2.7	2.4	6.0	protein WSI724 [Oryza sativa]
OV2_29_E11_A002	1.0	3.3	2.4	10.3	Protein translation factor SUI1 homolog [Z mays]
DG1_85_D01_A002	1.8	5.7	3.7	9.1	Putative ripening-related protein [Vitis vinifera]
IP1_2_A04_A002	0.6	0.9	1.2	0.9	Putative ATP phosphoribosyl transferase [Oryza sativa]
OV1_24_G11_A002	n/a	3.0	2.0	2.3	Putative histone H2A [Oryza sativa]
OV2_31_D03_A002	0.5	1.6	1.1	1.6	Protein disulfide isomerase precursor [Zea mays]
WS1_75_B02_A002	0.7	1.1	1.1	1.3	Biotin synthase (EC 2.8.1.6) [Arabidopsis thaliana]
WS1_74_C10_A002	1.6	5.9	0.1	9.8	Putative ripening-related protein [Vitis vinifera]
DSAF1_11_H04_A011	2.1	4.7	4.1	5.1	Glucose-6-phosphate 1-dehydrogenase [T. pallidum]

Table 8. Continued.

Clone name	3S	27S	3r	27r	Annotation
EM1_11_A05_A002	15.2	14.6	123.2	22.8	probable Na+/H+ antiporter [Arabidopsis thaliana]
PH1_2_C06_A002	0.6	1.9	1.8	2.1	Putative receptor kinase [Oryza sativa]
DG1_21_D02_A002	1.1	1.3	1.4	1.5	MAP kinase-like protein [Oryza sativa]
DSBF1_8_A02_A010	12.1	35.4	7.6	23.5	Oxysterol-binding protein-like [Arabidopsis thaliana]
WS1_37_D03_A002	2.1	4.6	4.6	5.4	aconitate hydratase (EC 4.2.1.3) [Lactococcus lactis]
IP1_69_F01_A002	0.5	1.9	1.4	1.5	Caffeoyl CoA O-methyltransferase [Zea mays]
RHIZ2_8_C04_A003	1.4	0.7	1.8	3.5	Osmotin-like protein [Benincasa hispida]
OV2_28_A11_A002	0.8	1.5	1.1	0.7	putative ABC transporter family protein [Oryza sativa]
PIC1_51_F08_A002	0.4	0.7	0.7	0.7	serine/threonine kinase-like protein [Oryza sativa]
LG1_264_F01_A002	0.9	0.8	1.9	1.0	Plasma membrane integral protein ZmPIP1-4 [Zea mays]
DSAF1_33_E06_A011	6.6	21.0	10.3	35.3	Calmodulin-like protein [Pennisetum ciliare]
WS1_22_F04_A002	4.3	21.0	7.4	119.4	S-adenosylmethionine decarboxylase 2 [O sativa]
PIC1_68_C06_A002	3.3	76.2	13.4	328.7	serine/threonine phosphatase type 2c [S stapfianus]
DSBF1_23_A06_A010	2.2	10.5	4.9	27.6	probable lipase [Zea mays]
WS1_68_C06_A002	n/a	n/a	n/a	n/a	protein WSI724 [Oryza sativa]
WS1_76_G06_A002	1.4	10.7	4.1	39.1	probable lipase [Zea mays]
DG1_31_C05_A002	9.6	29.0	8.7	27.4	Hypothetical 32.9 kDa protein [Arabidopsis thaliana]
WS1_30_B10_A002	2.4	10.6	5.9	27.6	Putative glycine and proline-rich protein [S. stapfianus]
WS1_75_C12_A002	7.2	34.9	27.2	111.5	ABA-responsive protein homolog T31B5.20 [A. thaliana]
DSAF1_3_E07_A011	2.3	9.4	3.9	8.7	trehalose-6-phosphate synthase homolog [A thaliana]
FM1_15_B03_A003	2.1	11.5	5.2	24.1	probable lipase [Zea mays]
PIC1_69_G10_A002	0.4	0.9	0.7	0.8	GTP binding protein-like [Arabidopsis thaliana]
PIC1_69_F10_A002	0.8	0.7	1.6	0.4	Putative beta-glucosidase homolog [Oryza sativa]
WOUND1_54_C01_A002	0.6	0.8	0.9	0.7	probable RING zinc finger protein [A. thaliana]
DSBF1_2_B09_A010	n/a	n/a	n/a	n/a	beta-D-galactosidase
RHIZ2_44_C02_A003	2.6	15.2	4.0	9.1	Senescence-associated protein 5 [Hemerocallis cultivar]
DSBF1_10_G06_A010	0.9	1.2	1.0	0.7	Putative zinc-finger helicase [Oryza sativa]

Table 8. Continued.

Clone name	3S	27S	3r	27r	Annotation
DSAF1_2_C03_A011	2.6	8.3	12.0	52.2	Early drought induced protein [Oryza sativa]
PH1_17_D07_A002	1.4	1.3	1.2	1.2	Protein kinase-like protein [Oryza sativa]
PI1_95_F07_A002	0.8	0.8	1.0	2.4	Putative fatty acid condensing enzyme CUT1 [O sativa]
DSBF1_27_B07_A010	n/a	n/a	n/a	n/a	Thioredoxin-like protein TXL [D. melanogaster]
LG1_206_E08_A002	2.1	1.5	3.3	3.4	omega-3 fatty acid desaturase (EC 1.14.99.-) [Z mays]
DSAF1_80_G10_A011	1.3	1.0	1.6	0.8	Putative tetratricopeptide repeat protein [Oryza sativa]
PIC1_69_A07_A002	5.9	17.2	4.1	10.9	P0005A05.30 protein (P0482C06.13 protein) [O sativa]
PIC1_72_F04_A002	6.3	13.8	6.9	5.0	dehydration-induced protein (ERD15) [A thaliana]
EM1_78_E08_A002	0.3	8.7	0.1	3.2	Thiazole biosynthetic enzyme 1-2 [Z mays]
WS1_21_C03_A002	0.7	12.3	1.7	9.1	unnamed protein product [Zea mays]
WS1_68_C01_A002	0.3	13.8	0.3	3.9	Thiazole biosynthetic enzyme 1-2 [Z mays]
OV2_28_A09_A002	1.4	0.5	1.2	0.6	Monodehydroascorbate reductase [Z. aethiopica]
LG1_334_C02_A002	0.9	0.7	0.8	0.4	Putative steroid membrane binding protein [Oryza sativa]
LG1_223_C04_A002	n/a	n/a	n/a	n/a	D-Lactate dehydrogenase [Vibrio vulnificus]
RHIZ2_23_A10_A003	2.0	2.3	1.2	0.9	Cellulose synthase-8 [Zea mays]
EM1_18_A08_A002	0.8	0.3	0.6	0.2	omega-3 fatty acid desaturase [Glycine soja]
PIC1_21_G11_A002	0.6	1.6	1.4	2.5	glucose-6-phosphate/phosphate-translocator [P .sativum]
DSBF1_25_H11_A010	2.1	5.8	7.1	7.8	Solute carrier family 6 [R. norvegicus]
WS1_23_A06_A002	5.9	17.9	1.5	4.8	SMCP-2 [Homo sapiens]
WS1_94_G04_A002	1.0	0.4	1.1	0.4	Hypothetical protein [Arabidopsis thaliana]
PIC1_42_F06_A002	1.0	3.7	1.1	3.3	Polyubiquitin [Sporobolus stapfianus]
WS1_35_D11_A002	2.7	2.0	1.6	1.4	AT07117p [Drosophila melanogaster]
WS1_34_G11_A002	0.7	0.5	0.5	0.2	similar to carboxylesterase [Oryza sativa]
WS1_1_H04_A002	2.0	3.9	2.1	2.7	OsNAC6 protein [imported] [Oryza sativa]
PIC1_69_A04_A002	0.5	0.9	0.8	1.5	Receptor-like kinase [Hordeum vulgare]

Table 8. Continued.

Clone name	3S	27S	3r	27r	Annotation
DSBF1_27_B05_A010	4.0	225.7	1.5	86.9	pumilio-family RNA-binding protein [Arabidopsis thaliana]
PI1_42_B03_A002	0.9	0.3	0.8	0.2	cellulase E-4 [Thermobifida fusca]
WS1_23_D08_A002	n/a	n/a	n/a	n/a	Putative extensin-like protein [Oryza sativa]
FM1_12_C07_A003	1.1	1.0	1.0	0.7	Superoxide dismutase [Cu-Zn] 4A [Zea mays]
PI1_48_H12_A002	0.6	0.8	2.0	6.1	Oxidase [Oryza sativa]
HS1_67_B11_A012	0.8	0.5	0.8	1.9	dipeptide ABC transporter [H. pylori]
DSAF1_17_A07_A011	2.2	126.0	2.5	32.4	Putative fruit-ripening protein [Oryza sativa]
ABA1_28_G10_A012	n/a	n/a	n/a	n/a	UDP-glycose:flavonoid glycosyltransferase [V mungo]
PI1_46_G09	0.8	25.2	2.9	98.7	WSI76 protein
WS1_101_E03	3.4	10.6	2.0	6.4	similar to protein tyrosine kinase 2
OV2_20_D07	1.0	4.3	1.5	3.3	Putative trehalose-6-phosphate phosphatase [O sativa]
IP1_9_G08	1.5	2.2	18.7	3.7	DREBP 3 [L esculentum]

Cold Specific Up-Regulated Genes

Cold and drought can both cause water deficit and the accumulation of ABA. Therefore, cold-induced genes are modulated in an ABA-dependent or independent manner. Therefore, genes modulated by cold treatment were tested for response to PEG, and ABA treatment. Table 9 shows that 18/69 genes modulated more than 2-fold by only cold treatment did not respond to ABA or PEG treatment.

Table 9. Genes responding selectively to low temperature.

Clone name	Stress	3S	27S	3 r	27 r
RHIZ2_16_C11_A003	**Cold**	0.91	0.73	2.23	1.19
	PEG	0.59	0.26	0.48	0.24
	ABA	0.36	0.19	0.72	0.30
Glyceraldehyde-3-phosphate dehydrogenase					

Table 9. Continued.

Clone name	Stress	3S	27S	3 r	27 r
LG1_225_B11_A002	**Cold**	2.94	9.25	2.04	7.37
	PEG	0.90	0.79	0.23	0.69
	ABA	1.58	1.50	1.57	1.74
	Amino acid transport protein [A. thaliana]				
DG1_39_E03_A002	**Cold**	2.03	1.02	1.76	0.66
	PEG	0.69	0.21	0.18	0.07
	ABA	0.90	0.02	0.31	0.01
	Putative aquaporin (Fragment) [Oryza sativa]				
OX1_55_H12_A002	**Cold**	2.25	11.76	11.43	32.93
	PEG	1.55	1.55	0.40	0.24
	ABA	1.40	0.51	1.28	0.97
	Glycine and proline-rich protein [S. commune]				
WOUND1_15_H04_A002	**Cold**	7.59	20.38	4.63	15.63
	PEG	0.62	0.86	0.17	0.30
	ABA	0.54	0.88	0.95	1.09
	DREB2A [A. thaliana]				
DSAF1_13_D12_A011	**Cold**	4.02	19.73	5.41	17.19
	PEG	1.10	0.93	1.22	0.34
	ABA	1.07	0.92	0.77	0.89
	unnamed protein product [Zea mays]				
LG1_228_A12_A002	**Cold**	2.36	11.81	5.17	7.91
	PEG	0.87	1.05	1.34	0.44
	ABA	0.46	0.68	1.04	0.79
	Senescence-associated protein 5 [H.cultivar]				
DSAF1_11_H04_A011	**Cold**	2.15	4.68	4.11	5.10
	PEG	0.93	0.47	1.99	0.40
	ABA	0.61	0.59	0.76	0.73
	Hypothetical protein				
EM1_11_A05_A002	**Cold**	15.25	14.60	123.17	22.78
	PEG	0.37	0.29	0.79	0.63
	ABA	0.79	0.34	2.55	0.38
	Hypothetical protein				
DSBF1_8_A02_A010	**Cold**	12.07	35.43	7.56	23.49
	PEG	1.41	1.11	1.12	0.22
	ABA	0.91	0.65	0.41	0.50
	Oxysterol-binding protein-like [A thaliana]				
WS1_37_D03_A002	**Cold**	2.13	4.62	4.64	5.42
	PEG	1.28	0.89	1.34	0.44
	ABA	0.72	0.71	0.81	1.03
	Hypothetical protein [O. sativa]				
DSAF1_33_E06_A011	**Cold**	6.58	21.05	10.31	35.30
	PEG	0.87	0.70	1.29	0.24
	ABA	0.64	0.43	0.56	0.49
	Calmodulin-like protein [Pennisetum ciliare]				

Table 9. Continued.

Clone name	Stress	3S	27S	3 r	27 r
PIC1_69_A07_A002	**Cold**	5.93	17.16	4.06	10.88
	PEG	0.48	0.74	0.15	0.32
	ABA	0.57	0.88	0.78	1.22
	P0005A05.30 protein [O sativa]				
RHIZ2_23_A10_A003	**Cold**	2.01	2.31	1.23	0.86
	PEG	0.34	0.41	0.33	0.37
	ABA	0.83	0.56	0.45	0.15
	Cellulose synthase-8 [Zea mays]				
IP1_9_G08	**Cold**	1.53	2.18	18.67	3.67
	PEG	0.81	1.21	0.64	1.02
	ABA	0.55	0.75	0.51	0.85
	DBF1 [L esculentum]				
PH1_2_C06_A002	**Cold**	0.61	1.93	1.77	2.14
	PEG	n/a	0.96	0.17	0.33
	ABA	1.01	1.14	0.05	0.04
	Putative receptor kinase [Oryza sativa]				
WS1_68_C01_A002	**Cold**	0.31	13.78	0.25	3.95
	PEG	0.89	0.83	0.55	0.87
	ABA	1.06	1.87	0.48	0.76
	Thiazole biosynthetic enzyme 1-2 [Z mays]				
LG1_206_E08	**Cold**	1.52	4.02	3.30	3.44
	PEG	0.27	0.44	1.02	1.09
	ABA	0.41	0.57	0.32	0.82
	omega-3 fatty acid desaturase FAD8 [Z mays]				

CONCLUSION

Of the ~12,000 genes analyzed on microarrays, exposure of plants to 20% PEG (drought stress) altered the expression of ~10% and transfer of plants to 4 °C (cold stress), modified the expression of ~8% of the genes. Previously identified drought-induced genes such as dehydrin, LTP and a phosphatase as well as unknown genes are up-regulated. Under cold stress, cold-inducible genes such as WSI, *erd*, and LEA as well as genes involved in signal transduction, lipid metabolism, transporter, and carbohydrate metabolism are induced. Some genes such as serine/threonine phosphatase, ABA response protein, lipase, and WSI76 vary in the kinetics of gene induction in response to low temperature. The expression of 69 genes modulated at least 2-fold from microarray was confirmed by qRT-PCR. 18 genes of these genes were modulated by cold but not by ABA and PEG treatment. This research provides the starting point for detailed analysis and comparison of ABA, water deficit, and cold modulated gene networks in sorghum.

DISCUSSION

During the course of this study, I identified cold inducible genes in *Sorghum*. The function of several of the cold-induced genes was identified through Blast analysis can be classified depending on functions of the encoded proteins. Membrane proteins such as aquaporin, hexose transporter and ion transporters are involved in water, sugar and ion movement. Proteins involved in protection from reactive oxygen species such as thioredoxin and glutathione *S*-transferase act as detoxification enzymes. LEA proteins, osmotin, and leucine rich repeat proteins play a role in protecting macromolecules and stabilizing membranes. Proteins such as glycine and proline-rich proteins, hydroxyproline-rich glycoprotein, carbohydrate-related proteins are involved in osmolyte biosynthesis. Regulatory proteins such as serine/threonine phosphatase, calmoduline-like proteins and the OsNAC6 protein are involved in stress-induced gene expression and the regulation of signal transduction in ABA-dependent and ABA-independent pathways.

I confirmed and further quantified the expression of numerous cold modulated sorghum genes by qRT-PCR. Several transcription factors play a role in regulating plant responses to abiotic stresses. The dehydration responsive element binding proteins (DREB) are important transcription factors involved in response to abiotic stresses. DREB1A,B,C (CBF3,1,2) and DREB2 are involved in two different signal transduction pathways although they bind to the C-repeat CRT/dehydration responsive element (DRE) motif on the promoter of cold and dehydration responsive genes. DREB1 in

Arabidopsis was expressed at low temperature through ABA-independent pathway, but not by dehydration and high salt stress. In contrast, DREB2 was induced by dehydration through ABA-independent pathway, but not by low temperature (19). C-repeat-binding factor 4 (CBF4) was induced under dehydration through the ABA-dependent pathway (32). In this study, DREB2A and CBF1 were induced by cold, but not by ABA and PEG. Therefore, DREB may be regulated by different signal transduction pathway in *Sorghum* and *Arabidopsis*. The expression of CBF1 in Sorghum showed more induction at 3h time point than at 27h time point like *AtDREB1B* at roots exposed to cold.

There are 131 *abc* transporter gene family members in *Arabidopsis thaliana*. Nonetheless, little is known about the function of ABC transporters in abiotic stresses. The *Sptur2* was induced by ABA, cold and salinity. The *Ospdr9*, PDR-type ATP-binding protein in rice was induced by polyethylene glycol and salt but not by cold and heat (33). In this study I found that one of ABC transporters in sorghum was induced by cold but not by PEG and ABA.

The alteration of lipid profiles in plasma membrane is important for cold resistance. Bohn *et al.*, (34) analyzed changes in plasma membrane lipids from non-acclimated, cold-acclimated and ABA-acclimated winter wheat. In cold-acclimated wheat, the most significant change was the increase of the ratio of free sterols/glycolipids. Compared to cold-induced effects, ABA-acclimated wheat did not show much change in free sterols. Sterols as a subgroup of steroids play a role in modulating membrane rigidity and fluidity. Membrane sterols are involved in signal transduction and vesicular trafficking. Yang (35) reported that oxysterol binding proteins

serve as nonvesicular sterol transport. In this study, oxysterol-binding protein was highly induced by cold.

Fatty acid desaturases are required for the conversion of saturated fatty acids to unsaturated fatty acids. The changes in fatty acid composition can be induced by abiotic stresses such as drought, salt and cold. The content of linolenic acid (18:3) was reduced under drought and salt in pea, wheat, and soybean. On the contrary, the amount of linolenic acid was increased in relation to cold and freezing tolerance (36). Cold stress can cause the phase transition of lipids which in turn depends in part on the ratio of saturated to unsaturated fatty acids. Omega-3 fatty acid desaturases utilize acyl groups bound to phosphatidylglycerol and phospholipids as substrates. Cyanobacteria desA using glycerolipids is responsible for chilling tolerance (37). Transgenic *Arabidopsis* overexpressing a chloroplast-specific omega-3 fatty acid desaturase under a cold-inducible promoter (*cor* 15a) showed cold tolerance (38). Results from sorghum microarray experiments showed that omega-3 fatty acid desaturase was induced under only cold stress.

Diverse environmental stresses such as light, hormones, pathogens and drought induce changes in intracellular calcium (Ca^{2+}) levels. Calmodulin-like proteins (CML) that bind Ca^{2+} can detect the level of Ca^{2+} changes and undergo calcium-mediated signal transduction by interaction with target proteins (39). In *Arabidopsis*, 50 calmodulin-like proteins are predicted and the functions of most proteins are unknown. Delk *et al*., (40) identified that CML24 binds Ca^{2+} and the level of CML24 transcripts is increased up to 15-fold in response to ABA, hydrogen peroxide, indole-3-acetic acid (IAA), heat and

cold. In the current study, a calmodulin-like protein was highly induced by cold but not by PEG and ABA.

Cellulose is the most abundant polysaccharide constituent of the cell wall. Cellulose synthase catalytic subunits (CesA4, 7 and 8) are required for secondary cell wall. AtCesA4, 7 and 8 mutants were unable to endure the negative water pressure due to reduced cellulose content. Mutants, *lew2-1* and *lew2-2* showing leaf-wilting phenotype were screened and *LEW2* gene was an allele of *AtCes8* gene. Chen *et al*., (41) from *LEW2* gene study suggested cellulose synthesis was involved in plant response to drought and osmotic stresses. Nogueira *et al*., (42) analyzed gene expression of sugarcane exposed to cold for 3 to 48 hours. Cellulose synthase was one of 20 novel cold inducible genes reported and might act as a barrier against ice formation for cold stress. A cold inducible sorghum cellulose synthase gene from was identified in this study.

ABA controls drought-, and cold-inducible gene expression indicating that stress-inducible genes are regulated in ABA-dependent and -independent manners. Interestingly, many cold-inducible genes are regulated by an ABA-independent pathway unlike drought-inducible genes induced by ABA-dependent pathway (22). DRE/CRT is a major cis-acting element for regulating ABA-independent gene expression in response to drought and cold. Overexpression of DREB1/CBF driven by the 35S CaMV promoter in transgenic plants showed increased tolerance to drought and cold stress (26). cDNA and GeneChip microarray studies identified more than 40 genes downstream of DREB1/CBF suggesting the existence of the DRE/DREB regulon (27). Surprisingly, some of those genes expressed in transgenic plants did not contain DRE motif on their

promoters suggesting some genes were classified into indirect downstream genes in response to cold stress.

Galactinol synthase (GolS) is a stress-inducible gene that functions in the accumulation of the osmoprotectants, galactinol and raffinose (43). Interestingly, SbGolS1 was induced by cold stress, but SbGolS2 was induced by drought and ABA stresses in this study. Taji *et al*. (44) reported that AtGolS3 was induced by only cold stress. On the contrary, AtGolS1, and 2 were induced by drought and high salinity stresses, but not by cold stress. DREB1A-overexpressing plants showed an increase of AtGolS3 expression with the increased raffinose levels and might improve freezing tolerance.

From this microarray study, I identified numerous stress inducible genes. Some of them were induced by cold stress but not ABA or dehydration. This information will allow future analysis of promoter cis-element modules that are associated with cold inducible genes in sorghum.

REFERENCES

1. Buchnan BB, Gruissem WG, Jones RL: **Biochemistry & Molecular Biology of Plant.** *American Society of Plant Physiologists* 2000, pp501-503.

2. Seki M, Narusaka M, Abe H, Kasuga M, Yamaguchi-Shinozaki K, Carninci P, Hayashizaki Y, Shinozaki K: **Monitoring the expression pattern of 1300 Arabidopsis genes under drought and cold stresses by using a full-length cDNA microarray.** *Plant Cell* 2001, **13**:61-72.

3. Wang H, Datla R, Georges F, Loewen M, Cuter AJ: **Promoter from *kin1* and *COR 6.6,* two homologous *Arabidopsis thaliana* genes: transcriptional regulation and gene expression induced by cold, ABA, osmoticum and dehydration.** *Plant Mol Biol* 1995, **28**:605-617.

4. Stockinger EJ, Gilmour SJ, Thomashow MF: **Arabidopsis thaliana CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to cold and water deficit.** *Proc Natl Acad Sci USA* 1997, **94**:1035-1040.

5. Gilmour SJ, Zarka DG, Stockinger EJ, Salazar MP, Houghton JM, Thomashow MF: **Low temperature regulation of the Arabidopsis CBF family of AP2 transcriptional**

activators as an early step in cold-induced *COR* gene expression. *Plant J* 1998, **16**:433-442.

6. Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF: ***Arabidopsis* CBF1 overexpression induces *COR* genes and enhances freezing tolerance.** *Science* 1998, **280**:104-106.

7. Gilmour SJ, Sebolt AM, Salazar MP, Everard JD, Thomashow MF: **Overexpression of the Arabidopsis CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation.** *Plant Physiol* 2000, **124**:1854-1865.

8. Thosmashow MF: **Role of cold-responsive genes in plant freezing tolerance.** *Plant Physiol* 1998, **118**:1-7.

9. Jaglo KR, Kleff S, Amundsen KL, Zhang X, Haake V, Zang JZ, Deits T, Thomashow MF: **Components of the *Arabidopsis* C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species.** *Plant Physiol* 2001, **127**:910-917.

10. Heish TH, Lee JT, Yang PT, Chiu LH, Charng YY, Wang YC, Chan MT: **Heterology expression of the Arabidopsis C-Repeat/dehydration response element**

binding factor 1 gene confers elevated tolerance to chilling and oxidative stresses in transgenic tomato. *Plant Physiol* 2002, **129**:1086-1094.

11. Schena M, Shalon D, Davis RW, Brown PO: **Quantitative monitering of gene expression patterns with a complementary DNA microarray.** *Science* 1995, **270**:467-470.

12. Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T: **Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray.** *Plant J* 2002, **31**:279-292.

13. Kreps J, Wu Y, Chang H-S, Zhu T, Wang X, Harper JF: **Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress.** *Plant Physiol* 2002, **130**:2129-2141.

14. Zhang X, Fowler SG, Cheng H, Lou Y, Rhee SY, Stockinger EJ, Thomashow MF: **Freezing-sensitive tomato has a functional CBF cold response pathway, but a CBF regulon that differs from that of freezing-tolerant Arabidopsis.** *Plant J* 2004, **39**:905-919.

15. Wang H, Miyazaki S, Kawai K, Deyholos M, Galbraith DW, Bohnert HJ: **Temporal progression of gene expression responses to salt shock in maize roots.** *Plant Mol Biol* 2003, **52**:873-891.

16. Kawasaki S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith D, Bohnert HJ: **Gene expression profiles during the initial phase of salt stress in rice.** *Plant Cell* 2001, **13**:889-905.

17. Oztur ZN, Talame V, Deyholos M, Michalowski CB, Galbraith DW, Gozukirmizi N, Tuberosa R, Bohnert HJ: **Monitoring large-scale changes in transcript abundance in drought- and salt-stressed barley.** *Plant Mol Biol* 2002, **48**:551-573.

18. Rabbani MA, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y, Yoshiwara K, Seki M, Shinozaki K, Yamaguichi-Shinozaki K: **Monitoring expression profiles of rice genes under cold, drought and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analysis.** *Plant Physiol* 2003, **133**:1755-1767.

19. Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K: **Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and**

low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell* 1998, **10**:1391-1406.

20. Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K: ***Arabidopsis* basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions.** *Proc Natl Acad Sci USA* 2000, **97**:11632-11637.

21. Jiang C, Iu B, Singh J: **Requirement of a CCGAC cis-acting element for cold induction of the BN115 gene from winter *Brassica napus*.** *Plant Mol Biol* 1998, **30**:679-684.

22. Yamaguchi-Shinozaki K, Shinozaki K: **Organization of cis-acting regulatory elements in osmotic- and cold-stress-responsive promoters.** *Trends in Plant Science* 2005, **10**:88-94.

23. Shinozaki K, Yamaguchi-Shinozaki K, Seki M: **Regulatory network of gene expression in the drought and cold stress responses.** *Current Opinion in Plant Biology* 2003, **6**:410-417.

24. Stockinger EJ, Gilmour SJ, Thomashow MF: **Arabidopsis thaliana CBF1 encodes an AP2 domain-containg transcription activator that binds to the C-repeat/DRE, a**

cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. *Proc Natl Acad Sci USA* 1997, **94**:1035-1040.

25. Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF: **Arabidopsis CBF1 overexpression induces COR genes and enhances freezing tolerance.** *Science* 1998, **280**:104-106.

26. Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K**: Improving plant drought, salt and freezing tolerance by gene transfer of a single stress-inducible transcription factor.** *Nat Biotech* 1999, **17**:287-291.

27. Maruyama K, Sakuma Y, Kasuga M, Ito Y, Seki M, Goda H, Shimada Y, Yoshida S, Shinozaki K, Yamaguchi-Shinozaki K: **Identification of cold-inducible downstream genes of the Arabidopsis DREB1A/CBF3 transcriptional factor using two microarray systems.** *Plant J* 2004, **136**:2734-2746.

28. Mullet JE, Klein RR, Klein PE: ***Sorghum bicolor* - an important species for comparative grass genomics and a source of beneficial genes for agriculture.** *Current Opinion in Plant Biology* 2002, **5**:118-121.

29. Buchanan CD, Klein PE, Mullet JE: **Phylogenetic analysis of 5'-noncoding regions from the ABA-responsive rab16/17 gene family of sorghum, maize and rice**

provides insight into the composition, organization and function of cis-regulatory modules. *Genetics* 2004, **168**:1639-1654.

30. Buchanan CD, Lim S, Salzman RA, Kagiampakis I, Klein RR, Cordonnier-Pratt M-M, Pratt LH, Klein PE, Mullet JE: **Transcriptome analysis of genes induced by ABA and osmotic stress in *Sorghum bicolor*.** *Plant Mol Biol* 2005, **58**:699-720.

31. Rodríguez EM, Svensson JT, Malatrasi M, Choi D-W, Close TJ: **Barley Dhn13 encodes a KS-type dehydrin with constitutive and stress responsive expression.** *Theor Appl Genet* 2005, **110**:852-858.

32. Agarwal PK, Agarwal P, Reddy MK, Sopory SK: **Role of DREB transcription factors in abiotic and biotic stress tolerance in plants.** *Plant Cell Rep* 2006, **21**:1263-1274.

33. Moons A: **Ospdr9, which encodes a PDR-type ABC transporter, is induced by heavy metals, hypoxic stress and redox perturbations in rice roots.** *FEBS Lett* 2003, **553**:370-376.

34. Bohn M, Luthje S, Sperling P, Heinz E, Dorffling: **Plasma membrane lipid alterations induced by cold acclimation and abscisic acid treatment of winter wheat seedlings differing in frost resistance.** *J Plant Physiol* 2006, **23**:1-11.

35. Yang H: **Nonvesicular sterol transport: two protein families and a sterol sensor?** *Trends Cell Biol* 2006, **16**:427-432.

36. Zhang M, Barg R, Yin M, Gueta-Dahan Y, Leikin-Frenkel A, Salts Y, Shabtai S, Ben-Hayyim G: **Modulated fatty acid desaturation via overexpression of two distinct omega-3 desaturases differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and plants.** *Plant J* 2005, **44**:361-371.

37. Wada H, Gombos Z, Murata N: **Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation.** *Nature* 1990, **347**:200-203.

38. Khodakvoskaya M, McAvoy R, Peters J, Wu H, Li Y: **Enhanced cold tolerance in transgenic tobacco expressing a chloroplast omega-3 fatty acid desaturase gene under the control of a cold-inducible promoter.** *Planta* 2006, **223**:1090-1100.

39. Reddy AS: **Molecular motors and their functions in plants.** *Int Rev Cytol* 2001, **204**:97-178

40. Delk NA, Johnson KA, Chowdhury NI, Braam J: **CML24, regulated in expression by diverse stimuli, encodes a potential Ca2+ sensor that functions in responses to**

abscisic acid, daylength, and ion stress. *Plant Physiol* 2005, **139**:240-253.

41. Chen Z, Hong X, Zhang H, Wang Y, Li X, Zhu JK, Gong Z: **Disruption of the cellulose synthase gene, AtCesA8/IRX1, enhances drought and osmotic stress tolerance in Arabidopsis.** *Plant J* 2005, **43**:273-283.

42. Nogueira FT, DeRosa VE Jr, Menossi M, Ulian EC, Arruda P: **RNA expression profiles and data mining of sugarcane response to low temperature.** *Plant Physiol* 2003, **132**:1811-1824.

43. Downie B, Gurusinghe S, Dahal P, Thacker RR, Snyder JC, Nonogaki H, Yim K, Fukanaga K: **Expression of a galactinol synthase gene in tomato seeds is up-regulated before maturation desiccation and again after imbibition whenever radicle protrusion is prevented**. *Plant Physiol* 2003, **131**:1347-1359.

44. Taji T, Ohsumi C, Iuchi S, Kasuga M, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K: **Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in Arabidopsis thaliana.** *Plant J* 2002, **29**:417-426.

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